
BLOOD

The Journal of Hematology

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The Journal of Hematology

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FOREWORD

to

Blood, The Journal of Hematology

MEDICINE is not a single science but depends on the integration of a number of sciences for the specific purpose of understanding the nature of disease as a natural phenomenon and the application of these to the prevention and treatment of ill health. As stated by Gabriel Andral of Paris a hundred years ago, hematology is a branch of the natural sciences for the study of the blood. Thomas Schwenicke in 1743 used this term and published a treatise on the subject. Andral is especially to be remembered as the first man to urge chemical examination of the blood, which led him in 1843 to the conclusion that there are primary blood diseases. Hematology is indeed to be considered as one of the branches of internal medicine.

Much knowledge and progressive interest has developed concerning the blood and its disorders in the past quarter century, so that now one may feel that a medical journal in English devoted to this subject is appropriate.

Medical journals should further knowledge and aid in disseminating it through the civilized world and in sifting the important pieces of work from the less significant on the basis of the presumably sound judgment of the editors. This journal should tell the reader of recent advances in the field of the blood and clarify those issues about which there is confusion. One hopes that papers will concern not only the blood cells but the development of knowledge concerning plasma fractions, the study of blood coagulation, and the like. The geneticist will wish for information relating to heredity in blood disease. Problems will concern infections with their varying leukocytic changes, the hematologic reactions to drugs as well as the different types of anemias, the leukemias, polycythemias, purpuras, and allied disorders. Questions concerning the use of heparin and dicumarol for reducing the coagulability of the blood, splenectomy, the proper use of transfusions of blood, the strange story of the Rh factor, the knowledge regarding vitamin K and prothrombin, and the consideration of hemolytic anemias are among the subjects which need further clarification. The bone marrow cells, the lymphatic tissues, as well as therapeutic developments will presumably command attention. The prevention of disease by study of the blood is important. Various industrial poisonings may be prevented by careful studies of the blood, and topics of this sort may be looked for.

The correct diagnosis is a prerequisite to efficacious treatment. Occasionally leukemia may be suspected by clinical manifestations although the blood cells may show relatively little alteration. On the other hand there may not be objective symptoms but the blood may yield full evidence of the disease. This sort of problem may receive elucidation in this journal.

Illustrations, especially in color, are important for the study of hematology. Often, however, language must serve to appeal to the senses when the objects themselves cannot be presented. The lability of children's blood is noteworthy,

and the hematopoietic equilibrium in infants is even less well established than in the child. One must appreciate the instability of the blood in younger individuals. Here leukocytosis may be more striking. Lymphocytosis develops with great ease in children, and such changes must be appreciated. Nucleated red cells may appear following a brisk hemorrhage where only polychromatophils would appear in an adult.

Medicine has to deal with human personality and human hopes and fears, and the physician will not succeed who does not appreciate the patient as a whole, including his mental anxieties. In medicine things spiritual and things material must both be appreciated in considering any one person. At present science has outrun philosophy, narrow specialization is the order of the day, and those interested in disorders of the blood should recall the importance of medicine as a whole. Science may deal with those aspects of human experience which are amenable to treatment by the scientific method, but in medicine one must deal with the human life, and one needs to adopt a practical attitude to the unknown.

It must not be forgotten that hematology is but one branch of general medicine and that physiology and the like bring much of significance to it. One must recall that the happiness of patients takes precedence, at least after diagnosis has been established. Real sympathy and concern for the patient are essential but must not interfere with one's determination to gain all possible information about the underlying disease. Clinical investigation takes a variety of forms; the aim should be to undertake fundamental problems. The best clinical investigator must be an able clinician, have wide interests, and understand human beings. He must possess an active, creative imagination and scientific curiosity, but the center of his activity must be the patient.

December 1945

GEORGE R. MINOT

BLOOD

A BRIEF SURVEY OF ITS CHEMICAL COMPONENTS AND OF THEIR NATURAL FUNCTIONS AND CLINICAL USES*

By EDWIN J. COHN, PH D

BLOOD has probably been studied more intensively in recent years than has any other tissue. This is partly because of the ancient tradition of its central position in the scheme of life, and partly because of the safety with which relatively large amounts can be withdrawn from or injected into man, in health or disease, for study or for therapy.

The availability of blood and its fluidity have given a deceptive impression, however, that it can be characterized as a whole. The brilliant investigations of Landsteiner at the turn of the century resulted in the recognition of a series of types of blood, and their number has increased as more and more observations, carefully carried out, have revealed greater and greater complexity. Besides the isoagglutinins which react with the A₁, A₂, or B agglutinogens, there are the various types of anti-Rh antibodies, anti-Rh₀, anti-Rh', anti-Rh'', and the late or blocking antibodies which have been recognized by the phenomenon of cell agglutination. We must, and do, characterize the whole blood in terms of these categories. To the best of our knowledge, however, the phenomenon of cell agglutination depends upon the surface of the red cell, certain environmental variables, and a very small group of plasma proteins, the isoagglutinins. The isoagglutinins are not the carriers of the immune, the osmotic, the respiratory, the coagulating, or the pressor properties of the blood. They are a genetic inheritance and normally represent less than a tenth of 1 per cent of the plasma proteins.

Although the importance of the red cell in concentrating within its envelope a series of specialized chemical reagents cannot be overemphasized, the specificity of cell agglutination, upon which blood grouping depends, does not involve the hemoglobin, the carbonic anhydrase, the hypertensinase, or the host of other enzymes whose functions within the red cells remain to be apprehended. Safety of red cell transfusion demands an ever increasing and more detailed knowledge of the agglutination phenomenon and of its control, and the steady growth of hematology

From the Department of Physical Chemistry, Harvard Medical School, Boston

* Our knowledge of blood has been accumulating for so many centuries that adequate references to the literature regarding each point referred to would occupy more space than this brief essay. Immunology and physiology, pharmacology and histology, physics, chemistry, medicine and surgery have all contributed to this knowledge. A partial bibliography regarding blood and blood derivatives is published in *Science in Progress*, Yale University Press, Fourth Series, 1945, pages 319-23. Recent studies upon separated plasma components which have led to clinical uses have been reported in the *Journal of Clinical Investigation*, volume 23, pages 417-66, 1944; volume 24, pages 662, 671, 698, 704, 793, and 820, 1945; and articles to be published shortly in the same journal in the *Journal of the American Medical Association*, volume 124, page 976, 1944; volume 126, pages 469, 674, 680, and 944, 1944; volume 127, page 144, 1945; volume 128, pages 1062 and 1088, 1945; and volume 129, page 270, 1945. In the *Bulletin of the New York Academy of Medicine*, Second Series, volume 21, page 202, 1945. In *Surgery*, volume 18, page 347, 1945; and in the *American Journal of the Medical Sciences*, volume 110, page 661, 1945.

is yielding such knowledge, as well as knowledge of the diverse products and processes upon which the multiple functions of the blood depend

Bacteriology has long recognized groups of substances in the blood which contribute to resistance to infection. Early workers distinguished between complement and specific antibodies on the basis of the greater thermostability of complement. Complement is, however, a complex of at least four different components, certain of which have been recognized and concentrated as euglobulins by precipitation near their isoelectric points. No one of the components of complement that have been thus separated represents as much as 1 per cent of the plasma proteins.

The group of globulins which have thus far been most clearly identified with the antibody functions of the blood, the γ -globulins, represent approximately 11 per cent of the plasma proteins. In this bundle of globulins are concentrated the antibodies to many of the common infectious agents to which man has been exposed. The γ -globulins will, therefore, differ not only in amount, but also in kind, from man to man, and from time to time in the same man, depending on the one hand upon his response to new antigens and on the other upon the complex system which controls the equilibrium between these and other plasma proteins and the tissues from which they come and in which they are stored. The pooling of the γ -globulins from a population offers a means of characterizing the state of immunity of that population. Even when injection of such antibodies is proven to have prophylactic value, however, as in the case of measles and infectious hepatitis, the immunization is short-lived, rather than lasting. Lasting immunity presumably depends upon the tissue mechanism of antibody production rather than upon the antibodies, which like all plasma proteins have but a short life in the blood stream and are released in the repeating pattern which reflects the antigens to which the body has been exposed.

Just as the immune bodies of the blood are considered as a protective mechanism against infection, so the substances involved in the coagulation of the blood may be conceived of as a protective mechanism preventing the loss of the circulating fluid of the body externally, or into tissue spaces. Most of the molecules in this mechanism are component parts of the plasma and are proteins. Since the elements of the solid blood clot must always be present, many of them exist in the flowing blood in a precursor state.

Physiology, on the basis of experiments and a developing theory, had postulated the existence of many substances involved in blood coagulation, which have in recent years been concentrated and purified. Among them is the structural element of the fibrin clot, the long rod-shaped fibrinogen molecule, which represents but 3 per cent of the plasma proteins. Were it present in larger amount it would so greatly increase the viscosity of the blood, by virtue of its asymmetric structure, as to impose a great burden on the heart.

Fibrinogen is transformed to fibrin by another protein, thrombin, but thrombin too exists in the blood in a precursor state as prothrombin. The production of prothrombin by the tissues is related to vitamin K, and this substance can be used to influence, within limits, the concentration of prothrombin in the blood stream. The transformation of prothrombin to the active state, thrombin, requires the

presence of calcium and of tissue lipoprotein, called thromboplastin by virtue of this property. Fibrinogen, vitamin K, prothrombin, thrombin, and at least certain tissue thromboplastins have now been prepared in sufficiently pure states so that we may look forward to very careful studies of the chemical transformations involved in the coagulation process and to the possibility that this increasing knowledge may also lead to increasing control.

Besides fibrinogen and prothrombin, the components necessary to form the clot, the blood stream carries a fibrinolytic enzyme capable of dissolving the clot. Moreover, there is some evidence that this enzyme also exists in the plasma in a precursor state requiring activation before its full proteolytic function can be performed. This proteolytic enzyme has thus far been less completely studied than fibrinogen and prothrombin and thrombin. Its possible value in therapy remains to be determined.

Another component of normal plasma related to the clotting of the blood has been recognized because of its absence in a group of individuals. The so-called antihemophilic globulin has been concentrated in a fraction of plasma (Fraction I in the system represented in figure 1) which also contains fibrinogen, but the evidence available suggests that this globulin is not fibrinogen. Its presence may be demonstrated in systems from which fibrinogen has been removed by thrombin or by heat coagulation. Although its separation as a chemical individual has not as yet been accomplished, the capacity of the antihemophilic globulin to bring the clotting time of hemophilic blood to normal, both *in vivo* and *in vitro*, indicates its potential value in the prophylaxis and therapy of this serious disease.

In the coagulation process, as in the action of complement, many substances, most of them proteins, thus play interrelated roles. Another group of substances appears to interact to produce widespread constriction of blood vessels. As in the case of the proteins involved in blood coagulation, our knowledge of these substances has heretofore been developed largely in physiology. Something of their chemical nature begins to be known, however, and their separation and concentration to any necessary degree of purity would appear to be possible. In this system also the component circulating in plasma is a precursor substance, protein in nature, hypertensinogen. Hypertensinogen is one of the α -globulins. It is somewhat labile *in vitro* and *in vivo* interacts with a substance, renin—liberated from kidney tissue (under certain conditions)—to yield a peptide called hypertensin*. Hypertensin appears to be the active substance which leads to constriction of the blood vessels. Its concentration in blood plasma is, however, rapidly reduced by an enzyme, hypertensinase. Hypertensinase is present in the plasma, but in far larger amount in the red blood cell.

What are the interrelations between enzymes of this kind in the plasma and in the red cell? Do they perform the same or supplementary functions? Are they the same chemically, or will they reveal chemical differences related to their specific functions? There is phosphatase in the red cells. Phosphatase and cholinesterase clearly have functions related to the phosphatides and cerebrosides, and the lipases and proteolytic enzymes to the lipids, lipoproteins, and proteins, of the blood stream.

* Various workers have given various names to all of these substances. e. g., hypertensin has also been called angiotonin.

The complex interrelations of the many substances constantly being built up and destroyed, as well as transported and released to, or absorbed from, the tissues have in some degree been revealed in recent years. Their detailed exploration and

PLASMA PROTEINS THEIR NATURAL FUNCTIONS AND CLINICAL USES AND SEPARATION INTO FRACTIONS

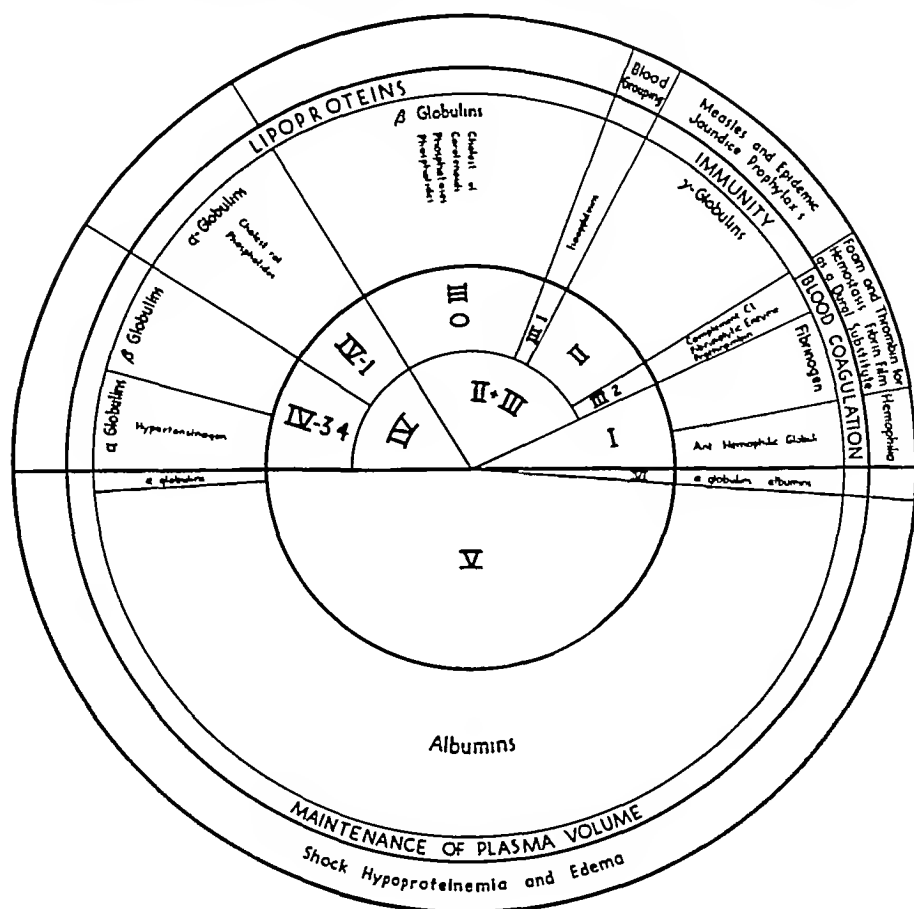


FIG 1

This figure is taken from that in *Science* (Vol 101 No 2612 pp 51-56 1945), somewhat modified to represent the most recent advances in the fractionation process. See also Methods for the Preparation of Anti-A Anti B and Anti Rh Isoagglutinin Reagents by J L Oncley, M Melin J W Cameron D A Richert, and L K Diamond (Annals of the New York Academy of Sciences In Press)

exploitation in the interests of chemical, and perhaps of clinical, control remains to be accomplished

Lipoproteins of many kinds have been separated from the plasma, and their investigation as chemical substances may well throw light on their nature and functions. Lipids have been found in more or less labile combination with α and β -globulins. The lipid components of these complexes are phosphatides, carote-

noids, fatty acids and cholesterol, and presumably other fatlike substances. The mode of combination is by no means proven, but it is of unquestioned significance that many of the fatty components of the plasma which had previously been believed to be free appear to be combined with protein. Moreover, their concentration under controlled chemical conditions has routinely yielded a series of characteristic lipoprotein fractions. The best estimate at the present time is that about a quarter of the plasma proteins are lipoproteins.

Three groups of the plasma proteins that have been prepared in relatively pure state are substantially free of lipids: fibrinogen, the γ -globulins, and the albumins. Certain α - and β -globulins, poor in lipid, have also been prepared (in Fraction IV-3,4) and studied both in the laboratory and in the clinic. The interrelation between these globulins, lipid-free and in combination with lipid, remains to be investigated.

Albumins are by far the most abundant of the plasma proteins. They are readily purified and crystallized. Free of carbohydrates, crystallized human serum albumin is composed of a small percentage of fatty acids in addition to the amino acids of which most proteins are constituted. The nature of the specific properties that these substances contribute to the albumins also raises interesting problems.

Albumins are the most symmetrical of the plasma proteins. Their solutions have therefore the lowest viscosity, a 25 per cent solution being iso-viscous with whole blood. The molecular weight of the albumins is half that of most globulins and one sixth that of fibrinogen. Only one in every hundred plasma protein molecules is fibrinogen, whereas two out of every three are albumin molecules. The albumins are responsible for most of the osmotic efficiency of the plasma and are thus largely responsible for the maintenance of blood volume. The use of albumin in the restoration of blood volume in shock and other conditions and as a diuretic agent in cirrhosis of the liver and nephrosis is discussed in a series of recent clinical papers.

The different highly specialized proteins of the plasma perform very different functions and are present in very different amounts. Thus the isoagglutinins represent less than one tenth of 1 per cent, each component of complement less than 1 per cent, prothrombin less than one tenth of 1 per cent, fibrinogen 3 per cent, the γ -globulins 11 per cent, the α - and β -globulins, of which 24 per cent are lipoproteins, represent 31 per cent, and the albumins about 55 per cent of the plasma proteins.

All the plasma proteins together, however, represent but 7 per cent of the normal plasma, or roughly 4 per cent of the blood. The amount of protein in the plasma thus is small in comparison with that in the red cell. Since roughly 30 per cent of the contents of the red cell is hemoglobin, or 12 to 14 per cent of the blood is hemoglobin, three to four times as much protein is present within the red cell as in the plasma.

Hemoglobin, like the other proteins of the blood stream, is highly specialized for the functions it performs. The importance of the respiratory function and the copiousness of this blood-red protein has led to a century of physiologic and clinical investigations, supplemented in more recent years by organic chemical studies of the structure of the iron-containing moiety, heme, which is the prosthetic group attached to the protein globin, and by physicochemical studies upon hemoglobin.

and its interactions especially with electrolytes, carbon dioxide, and oxygen. The atomic weight of oxygen is 16 and of iron 55. Each iron atom combines with an oxygen molecule, that is, two oxygen atoms. The iron, however, is part of the heme group with a combining weight close to 700. The heme is attached to the globin, each iron atom to a unit of 16,700 equivalent weight. Iron-containing proteins concerned with respiration in muscle, known as myoglobin, are of this size, but the hemoglobin of the blood stream has a molecular weight of at least 33,400 and generally of 66,800. In the former state it combines and transports two, in the latter four, oxygen molecules. The molecules devised by nature for the efficient transport of oxygen to the tissues thus have a mass roughly 500 times that of the oxygen to be transported.

Why is the hemoglobin of the blood stream contained within cell walls? The proteins which perform comparable respiratory functions in many forms of life are free in the fluid part of the blood. The proteins dissolved in the fluid part of the blood of animals rarely exceed 10 per cent, as contrasted with the 30 per cent of hemoglobin contained in the red blood cell. If this concentration of hemoglobin were free in the blood stream, it would bring about a redistribution of many components other than oxygen. It is the way of the body, or appears to be to those concerned with the study of its mechanism, to evolve processes which in enhancing the efficiency with which certain functions are performed do not inhibit others.

The function of transporting oxygen by the hemoglobin within the red cell involves a complex mechanism permitting maximum combination with oxygen in the lungs and release of oxygen in the tissues. As carbon dioxide increases, the hemoglobin unloads the oxygen which it has transported. Carbonic anhydrase accelerates and renders more efficient this process. In addition, the red cells contain other enzymes, including a catalase and a phosphatase. The presence of these enzymes suggests the nature of certain of the chemical reactions which are involved in the internal economy of these cells. These vital processes also appear to limit the life of the red blood cell, both in the blood stream and in the blood bank, under even the best methods of preservation at present known.

No other part of human blood has so significant a function in transporting oxygen to the tissues, in permitting the very great activity and high order of mammalian respiration. When, therefore, there is great blood loss from a wound, when a major operation is to be performed, or when the anemia which frequently occurs in convalescence from wounds must be combated, red blood cells must be supplied in amounts adequate to restore the oxygen-carrying capacity of the blood. In how many conditions red cells resuspended in media containing certain, but not necessarily all, of the plasma proteins and free of the iso-agglutinins will prove as valuable in therapy as whole blood remains to be determined. All of the components playing a role in the control of all of the functions performed by the cells and plasma may not be needed in prophylaxis or in therapy in all conditions, and those that are needed may have far greater value if made available in greater amount than their normal concentration in the blood or plasma.

It has proven possible to purify and concentrate certain of the plasma proteins, to prepare products from them, and to determine their clinical uses. Other products

derived from the cellular as well as the fluid part of blood can also be made available. Whether or not the natural products concentrated within the red cell can be as effectively employed as concentrates, of value in specific conditions, as those that are usually free in the plasma, can only be determined by careful, long-range experiments. All of these products may be separated, purified, and concentrated from the same blood. The chemist is fulfilling his function in developing methods for the preparation of derivatives which are more concentrated, stable, and specific agents than the blood from which they are derived. Starting with the assumption that every part of human blood performs an important natural function, we must make available in the postwar world, as we have in the war, as many as possible of its diverse cellular, protein, and fatty components, separated and concentrated as specific therapeutic agents, of value in different conditions, in the interests of the most effective and economical use by a society of the blood which it contributes.

PRIMARY CONGENITAL AND SECONDARY ACQUIRED SPLENIC PANHEMATOPENIA

B₃ CHARLES A. DOAN, M D , AND CLAUDE-STARR WRIGHT, M D

IN the consideration of human health and disease, the spleen has now become of much greater interest pathologically than physiologically. This is a distinct reversal of the situation even of a few years ago. That the spleen is nonessential at any age to the maintenance of life and health is amply attested by experimental extirpation of that organ in many animal species, and by the accumulated experience of emergency splenectomy for traumatic rupture of the normal human spleen.¹ Most of the recent progress, therefore, in our understanding of this enigmatic organ has been made through an increasingly critical study of human disease syndromes. The inferences suggested by the available histologic and physiologic data have become magnified, thereby, into an increased awareness of the importance of splenic pathology. Furthermore, if and when such pathology remains unrecognized in the human patient, chronic invalidism or acute, fatal hemoclastic crises may result.

In the interest of simplification and clarity, a correlation of the currently accepted knowledge relating to the spleen may be attempted diagrammatically (fig. 1). There are three rather obvious structural subdivisions in the spleen which serve as the natural basis for functional and pathologic considerations: (1) the vascular system, (2) the lymphoid system, and (3) the reticulo-endothelial system. Without attempting here to enter into a discussion of the controversial and conflicting evidence²⁻³ relative to the anatomical details of the sinusoidal circulation, it is quite evident that the smooth muscle in capsule and trabeculae, together with the vascular system of the splenic parenchyma, serves admirably as the structural basis for the reservoir function for blood cells and plasma, so well established by the classical experiments of Barcroft⁴ and others.⁵ Pathologically, there may be parenchymal congestion with splenic enlargement and excessive sequestration of plasma and cellular elements secondary to myocardial incompetence, bacterial toxemia, or hepatic cirrhosis with portal hypertension. The spleen is a lymphatic organ with typical lymph sinuses and follicles, the germinal centers of Fleming reflecting normal lymphopoiesis. The physiologic function or functions of the lymphocytes continue under investigation and discussion. It has become more obvious with each succeeding study that they are not simply precursors of other blood cell types, as originally hypothesized. Probably they have to do with endogenous protein metabolism, more specifically with globulin antibody elaboration in association with the reticulo-endothelial phagocytes.⁶⁻⁷ The characteristic, nonspecific, postinfective peripheral lymphocytoses, and the infiltrative phenomena in localized tubercle formation and chronic abscesses are the obvious pathologic evidence of the more subtle role played by these elements in the body's cellular and humoral

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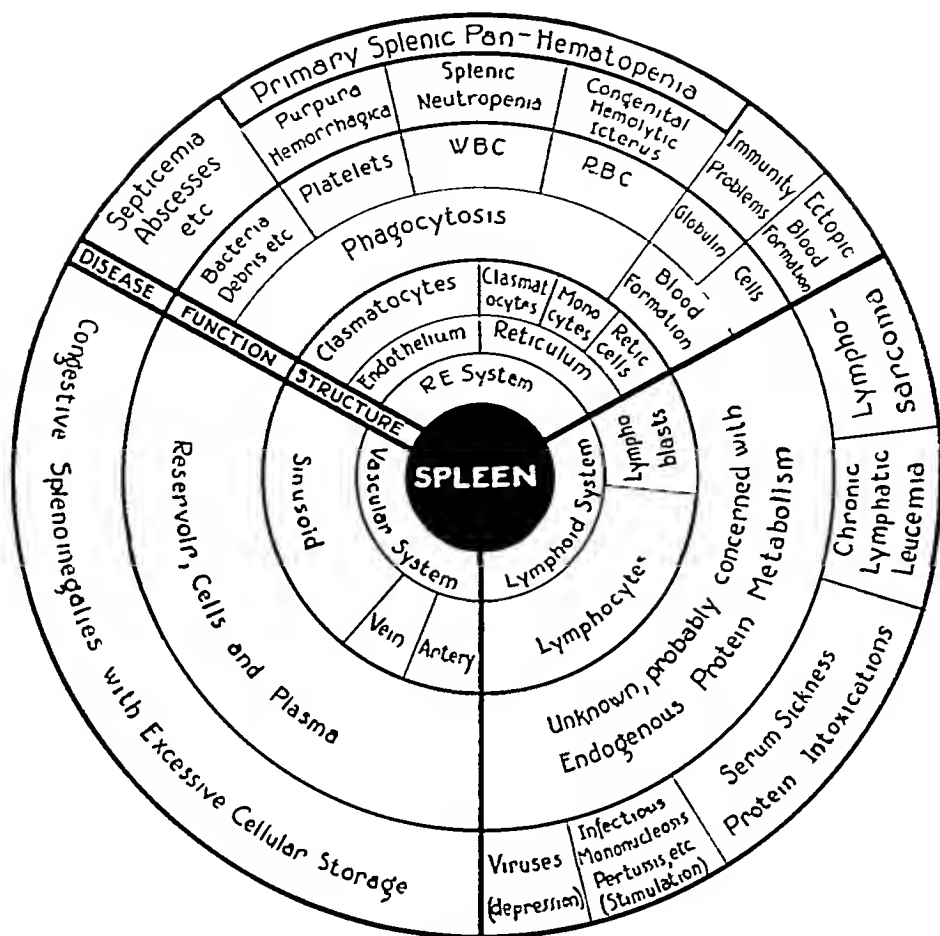


FIG. 1

defenses. The lymphopoietic foci in the splenic parenchyma reflect and parallel the general lymphocytic responses in the body, participating in the hyperplastic reactions in chronic lymphatic leukemia, infectious mononucleosis, serum sickness, and other types and varieties of protein sensitizations and intoxications. It is, however, within the reticulo-endothelial system of phagocytic cells that the principle mysteries of the pathologic role of the spleen in human disease probably reside. The R-E system of cells is made up of specific endothelia and reticulum cells endowed with special phagocytic capacities. The reticulum cells of the adult diffuse connective tissues behave as do primitive mesenchymal elements with embryonic potentialities. They give rise to other reticulum cells and to the primitive free cells from which monocytes differentiate and mature. The fixed endothelial cells, lining the vascular and lymphatic sinusoids, have a marked phagocytic capacity both in situ and as free, desquamated clasmatoctes⁵⁻⁹. Under appropriate circumstances, this focus of potentially developmental and embryonic-like mesen-

chymal tissue in the spleen may revert to a compensatory type of ectopic blood cell formation in the adult (myeloid metaplasia)

The normal spleen, thus, is an organ in which blood cell formation, blood cell sequestration, and blood cell destruction are balanced physiologic functions. If this be true, at any time a pathologic dysfunction in any one of these categories may develop with the appearance of a variety of clinical syndromes. It may also be conceded that a congenital accentuation or diminution in these physiologic functions might be expected occasionally, and that under certain circumstances, as the spleen becomes involved secondarily in a general constitutional disease process, a delicately balanced splenic equilibrium may be upset.

During the past fifteen years in our Hematologic Spleen Clinic we have observed a large variety of disturbances in the circulating blood cell equilibria, due to splenic dysfunction. This does not mean, of course, that the bone marrow and other organs may not also modify and contribute to the hematologic and clinical picture in a given syndrome. The definitive proof of the primacy of the splenic influence in any given instance is the favorable result of total extirpation of all splenic tissue, medical management having proved completely ineffective. When this test of surgery has been applied in acquired secondary, as well as in apparent primary splenic disease syndromes, the completeness and permanency of the resulting cellular and clinical re-equilibration furnish the ultimate criteria of the physician's diagnostic acumen.

The acute or chronic selective sequestration and destruction of red blood cells (congenital or acquired hemolytic icterus),¹⁰ platelets (thrombocytopenic purpura hemorrhagica),¹¹ and granulocytes (primary splenic neutropenia),¹² respectively, associated with a hyperplasia of highly phagocytic R-E cells, with or without demonstrable splenic enlargement, have been treated successfully by splenectomy. Definite, though secondary and usually asymptomatic, decreases in one or more of the circulating cell types not primarily involved have been noted in the previous communications dealing with each of the above as a separate clinical entity. Under such circumstances, it has been possible usually to observe a modified degree of abnormal clasmatocytic phagocytosis of the affected elements in supravital studies of the cellular detail of the freshly removed spleen.* In one of our patients with acute splenic neutropenia, for example (case 4¹²), there was an accompanying profound thrombocytopenia with clinical manifestations of purpura, without any appreciable hemolytic anemia, which emergency splenectomy completely and permanently corrected. In another chronic neutropenic syndrome in a 65 year old woman (case 3¹²), followed for more than a year before surgery, there developed under observation, secondary thrombocytopenic and anemic cellular and clinical manifestations, entirely absent when the patient was first seen with a selective neutropenia only. Our patients with active congenital hemolytic icterus (see fig 1¹⁰) more frequently than not have shown an associated moderate leukopenia and

* These observations are at variance with our own, which indicate an unusual inhibitory effect of an overactive spleen upon the bone marrow rather than one wholly due to unusual sequestration and phagocytosis within the spleen. Whatever the interpretation however, the two points of view coincide as to the reality of the pathologic syndromes cited and the effects of splenectomy. —EDITOR

thrombocytopenia, which were immediately relieved following successful splenectomy. The normoblastic marrow hyperplasia may have played a myelophthisic role in some of these patients, but a careful study of the fresh splenic parenchyma by the supravital staining technic provided direct evidence of increased granulocyte and thrombocyte inclusions along with the red cells in the numerous R-E phagocytes.*

The recognition of this erratic pathologic physiology of the spleen in these well known and clinically established primary entities led quite logically to a closer scrutiny of the patients referred to us with the peripheral blood picture and clinical symptoms and diagnosis of hypoplastic or aplastic anemia. Likewise, in certain general constitutional disease states involving the spleen more extensively than other organs, we have begun to note in recent years an instability in the functional balance of the spleen, which in extreme instances has resulted in an absolute lack of any discrimination between senile and mature circulating elements, with excessive phagocytosis of all essential cells passing through this organ. Certain selected cases will illustrate the extremes in this series of splenic phenomena.

CONGENITAL SPLENIC PANHEMATOPENIA

Case 1 Betty Jane, aged 14 years when first seen in this clinic, was born in Texas, Dec. 9, 1928, full term, weight 9 pounds, the first child of physically normal parents. During the first six months of extrauterine life she was breast fed, gained weight normally, and the mother noted no unusual symptoms. Toward the end of this period, however, the infant became irritable and began to refuse her feedings, her sleep became broken, she ceased to gain, and a marked pallor without jaundice was noted. Unexplained convulsive seizures, as many as six a day, developed with intermittent cyanosis, and transitory loss of consciousness. At this time, a pediatrician in San Antonio was consulted and a diagnosis of nutritional deficiency anemia was made. However, following four months on an optimum dietary regimen plus iron, the weakness was more marked and the pallor had become extreme. A second pediatrician was consulted in Austin, from whom we have the first definite blood findings, dated April 30, 1931: total WBC, 4,200, RBC, 1,210,000, hemoglobin, 4.5 Gm., differential white cell count, PMN 29 per cent, PME, 1 per cent, lymphocytes, 65 per cent, monocytes, 1 per cent. There were marked poikilocytosis, anisocytosis, and polychromatophilia of the red cells. A third pediatrician made many tests over a period of two years, trying out various treatments without any results. Blood transfusions were discussed but never employed. An x-ray examination of the chest showed a markedly dilated heart. Betty Jane was presented before the Southern Medical Association in 1937 as a diagnostic enigma and therapeutic problem. A hypoplastic anemia was assumed, though duration and other features were atypical. At 4 years of age she had measles, at 4½, pertussis, at 6, mumps, and at 9, chicken pox. There were occasional episodes of unexplained fever. She had a known allergic sensitization to wheat, with resulting nasal congestion. There had been distinct fluctuations in her state of well-being, varying with the degree and intensity of the anemic pallor. The menses had not yet started. She had had almost continuous difficulty with skin disorders, lesions of the toes and fingers, particularly, persisting for months despite the constant care of a dermatolo-

* See footnote, page 12.

gist. Her appetite remained extremely poor, insomnia persisted, with bed wetting regularly, and emotional instability resulted in frequent crying spells. Despite these handicaps, she indulged in limited swimming, skating, and dancing, though developing dyspnea quickly and requiring frequent long periods of rest. She led her classes in school through the years, though climbing school stairs had been physically exhausting.

Betty Jane was referred to our clinic October 13, 1943. There was marked pallor of skin and mucous membranes, the conjunctivae and nail beds were white. Temperature, 99, pulse, 105, regular, respirations, 25 per minute at rest, blood pressure systolic, 110, diastolic, 80 mm. hg. There was no general lymphadenopathy, thyroid gland, normal. The head, hair distribution, eyes, ears, nose, mouth, tongue, teeth, and throat were all normal. Secondary female sex characteristics were well developed and compatible with age. The lungs were clear to physical examination and fluoroscopy. The heart contour was slightly enlarged, the point of maximum intensity being in the fourth interspace in the left anterior axillary line, with a hemic apical systolic murmur, no diastolic murmurs. The spleen was palpable, not tender, 2 cm. below the left costal margin, descending on deep inspiration. The liver was not enlarged to physical examination, and there were no intra-abdominal masses. Genitalia normal, virginal, female, with beginning pubic hair development. The extremities were negative, no edema, no petechiae or ecchymoses. The neurologic examination was physiologic throughout.

The hematologic data (see fig. 2) on admission were as follows: WBC, 1,600, RBC, 1,030,000, hemoglobin, 3.5 Gm., reticulocytes, 3 per cent, platelets, 228,000 (normal 750,000), hematocrit, 10, MCV, 97 c. mm., MCH, 35.77, MCHC, 35 per cent, corrected erythrocyte sedimentation index, 0.2 mm. per min. (normal), erythrocyte fragility range, 412 — 341 per cent NaCl (normal), icterus index, 7, van den Bergh reaction, physiologic, supravital differential count of the white cells, polymorphonuclears, 36 per cent, eosinophiles, 18 per cent, lymphocytes, normal, small, 38 per cent, monocytes 8 per cent. Sternal marrow aspiration: supravital stains of grossly hyperplastic fresh tissue, revealed, myeloid:erythroid ratio of 4:1, neutrophilic myelocytes C 67 per cent, eosinophilic myelocytes C 20 per cent, lymphocytes 5 per cent, plasmatocytes 2 per cent, megakaryocytes 3 per cent. Erythroid series: normoblasts 30 per cent, late erythroblasts 20 per cent, early erythroblasts 22 per cent, megaloblasts 28 per cent, no foreign or atypical cell types seen. Other laboratory tests: basal metabolic rate plus 15 per cent, fasting blood glucose 95 mgm. per cent, blood urea nitrogen 11 mgm. per cent, phenolsulfonphthalein excretion 85 per cent in two hours, urinalysis, chemical and microscopic, within normal limits, prothrombin, initial concentration 54 per cent, subsequently 95 to 102 per cent. Serology: Wassermann and Kahn tests negative. Electrocardiogram showed sinus tachycardia only without evidence of axis deviation or ventricular preponderance. X-ray of chest and skeleton revealed no significant pathology.

The results of the *adrenalin test* are indicated on the chart (fig. 2). At the height of the splenic contraction the total white cell count was found to have risen from the base line of 1,450 to 10,250, the red blood cells from 1,300,000 to 1,700,000 per

cu mm, the hemoglobin from 5.3 to 6.5 Gm, the platelets from 313,000 to 911,000 per cu mm. The above laboratory data, when interpreted with the background of history and physical findings, served to incriminate the spleen as the major factor in the current syndrome, while minimizing the possible role played by other essential organs.

Splenectomy was, thereupon, advised and accepted, and on October 21, 1943, a 475 Gm spleen was successfully removed by Dr. Verne Dodd. There was no evidence of perisplenitis, and there were no adhesions. The liver was entirely normal in appearance, with thin, sharp margins, no scarring, and no evidence of portal hypertension. Adrenalin was injected directly into the splenic artery prior to pedicle ligation, with prompt visible contraction of this organ. Preoperatively the total white cell count was 2,100, polymorphonuclear leukocytes 798, immediately postoperatively 17,000, PMN 7,140, at 4:30 p.m. 23,000, RBC from 1,000,000 to 2,010,000 to 2,180,000, thrombocytes from 292,900 to 1,157,000 to 1,521,000, hemoglobin from 4.3 Gm to 6.3 to 7.8 Gm. Studies of the fresh splenic tissue in supravitality stained preparations showed normal lymph follicles, with increased granulocytes, mature red cells and thrombocytes, within scattered highly phagocytic clasmacocytes. There was diffuse endothelial hyperplasia of the splenic sinuses and considerable intra- and extracellular pigment and debris. There were 3 to 5 eosinophils in each oil immersion field. No increase in fibrous tissue was apparent, no hemopoiesis.

The convalescence was uneventful, and the patient was discharged to her home on the eighteenth postoperative day with 9,000 leukocytes, of which 3,780 were normal mature neutrophilic granulocytes, 3,330,000 red cells, with 9.7 Gm hemoglobin and 8.2 per cent reticulocytes. The clinical appearance and actions of this patient changed as dramatically as the laboratory data indicate.

On December 5, 1943, Betty Jane's mother reported: "I am happy to say she is looking and feeling better every day. Naturally, even her disposition seems changed. She literally skips, dances, and sings most of the time. I do have a new child, now. Again on October 6, 1944, approximately one year following splenectomy. Betty Jane is certainly greatly improved over a year ago. She has grown about 3½ inches and gained 21 pounds. She looks fine and seems to feel fine. For the first time in her life she is trying a regular course in physical education in school. She has absolutely no trouble with menstruation (first menstrual flow Jan. 30, 1944). Everything pertaining to it seems very normal. You might also be interested to know that for months now she has had no skin disorders. I am very happy over her condition. On May 22, 1945. I do know that Betty Jane is a different child since she had her operation. She feels much better and is full of fun and life. Her coloring seems to improve all the time and now she is acquiring a fine sun tan. Her height is now 5' 3¾" and her weight 126 pounds. We are very happy over her condition. At the present writing, two years postoperatively, there has been no further evidence of waning bone marrow efficiency. While the red cells have remained stabilized between three and four million for the most part and the hemoglobin between 9 and 11 Gm, the white cells and thrombocytes have persisted at high normal levels.

From the evidence, we have concluded that this individual has probably always experienced excessive splenic sequestration of normal circulating blood elements,

and that, beginning a few weeks after birth, objective signs of the resulting cellular deficit appeared and, with minor fluctuations, persisted to the point of semi-invalidism until this organ was removed fourteen years later. An almost complete cellular and physical re-equilibration followed immediately and has been maintained for at least two years, with a very favorable future prognosis.

ACUTE SPLENIC PANHEMATOPENIA

In addition to the chronic relapsing syndrome just described, splenic panhematopenia may occur as a relatively acute fulminant medical crisis.

Case 2 Mrs. D. K., aged 24 years, was admitted as a medical emergency to the Hematologic Service, University Hospital, 6 p.m., August 14, 1944, with a history of vague symptoms of generalized malaise and fatigability for six months, subacute manifestations for four weeks, climaxing in an acute fulminant crisis of seventy-two hours duration. Increasingly profound prostration with tachycardia, palpitation, dyspnea, precordial pain, marked pallor with mild fluctuating jaundice, during the preceding ten days, and nausea and vomiting for seventy-two hours were the presenting complaints. The past personal and family histories were entirely negative for any significant illnesses. The patient had been married only six months with no interruption of or irregularity in the menses. Except for clinical icterus plus extreme pallor, with evidence of recent weight loss and moderate tissue dehydration, the only other significant positive physical finding was a large mass in the upper left quadrant, which moved on respiration and had a readily palpable splenic notch. Temp. 101° , pulse 120, respirations 25, blood pressure 110/40. The referring physician had reported the following laboratory data obtained at 9 a.m. the day of admission: WBC, 3,750, PMN, 68 per cent, lymphocytes, 32 per cent, RBC, 1,450,000, hemoglobin, 4.6 Gm. Our own initial studies at 6 p.m. the same day were as follows: WBC, 2,100, PMN, 420, myelocytes C 210, total RBC, 820,000, hemoglobin, 2.8 Gm, reticulocytes, 69 per cent, platelets 16,000 (normal 750,000), M.C.V., 90 cu. microns, M.C.H., 30.77, M.C.H.C., 33 per cent, sedimentation index, 0.2 mm./minute, erythrocyte fragility range, 471 — 341 (normal 412 — 300), serology, negative. *Sternal marrow* aspiration revealed a grossly hyperplastic tissue with microscopic panhyperplasia of all normal marrow elements with normoblasts predominating. Myeloid:erythroid ratio, 1:4. Supravital differential: metamyelocytes neutrophilic 60 per cent, myelocytes C 78 per cent, myelocytes B 0.5 per cent, myelocytes A 0.5 per cent, myeloblasts 0.5 per cent, PMB 1 per cent, PME 6 per cent, lymphocytes 0.5 per cent, phagocytic clasmatoocytes 5 per cent, megakaryocytes 2 per cent, normoblasts 86.2 per cent, late erythroblasts 7.6 per cent, early erythroblasts 3.6 per cent, megaloblasts 2.6 per cent, there were many mitotic nuclear figures, particularly in the red cell series.

Emergency splenectomy was successfully accomplished by Dr. George Curtis five hours after admission, while intravenous glucose-saline was being administered. The release of cells was dramatic (fig. 3), the immediate pre- and postoperative hematologic data, uninfluenced by blood transfusion, being as follows: WBC, 2,100 to 4,900, RBC, 1,010,000 to 1,990,000, hemoglobin, 2.8 Gm. to 5.6 Gm, platelets, 16,000 to 114,000. The spleen weighed 1,400 Gm and measured 25 x 15 x 5 cm.

Supravital studies of the fresh splenic pulp showed increased numbers of highly phagocytic clasmotocytes, 5 to 6 per oil immersion field, extracellular hemosiderin, with both normoblasts and myelocytes C in moderate numbers. A liver biopsy and mesenteric lymph node were secured for histologic study, and both were entirely normal in cellular and connective tissue structure.

There were no complications, and the hematologic and clinical improvements paralleled each other so that by the twelfth postoperative day the patient was discharged by automobile to her home in another city, WBC, 7,050, RBC, 3,030,000, PMN, 68 per cent, PME, 4 per cent, lymphocytes, 20 per cent, monocytes, 7 per

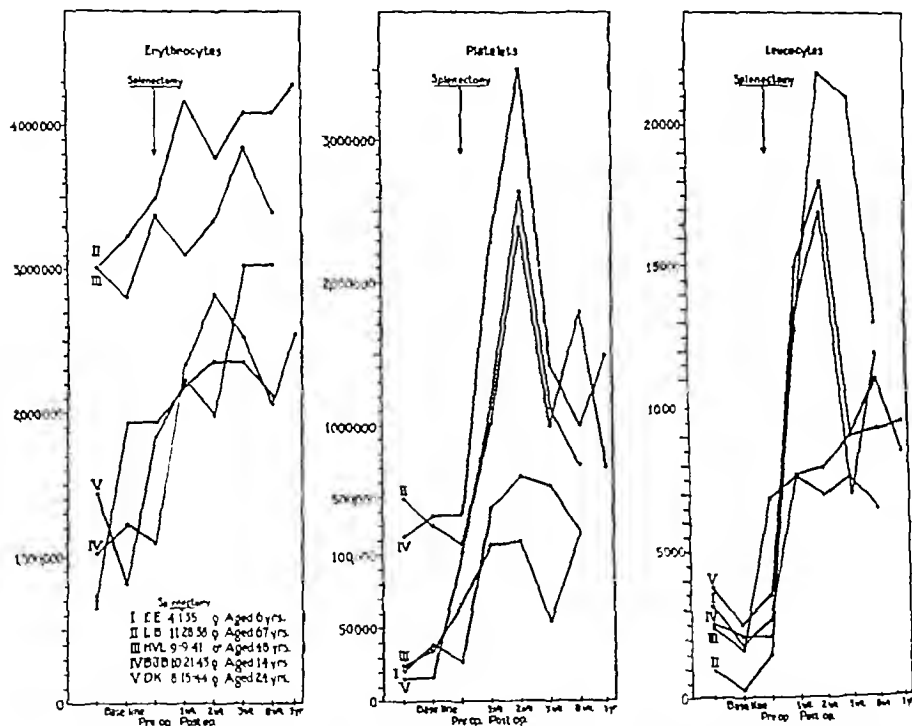


FIG 3 Summary of five cases of primary splenic panhematopenia which have been followed for a year or more, illustrating the recovery and sustained elevation of all of the depressed circulating blood elements following splenectomy.

cent, reticulocytes, 3.8 per cent, hemoglobin, 9.3 Gm, hematocrit, 29 per cent, platelets, 2,637,000.

While our preoperative historical inquiry failed to reveal any evidence of a familial basis for this acute crisis, it became possible during the convalescence of this patient to question and examine the immediate blood relatives. The high reticulocyte level, the increased erythrocyte fragility, the clinical jaundice, the size and gross appearance of the spleen, and the acute hemoclastic crisis, even in the absence of a generalized and uniform microcytosis, were all classical and typical of similar episodes, which we have seen and described in congenital hemolytic icterus.¹⁰ The father, one brother, and two sisters had essentially normal hemograms.

The mother, however, had a definite microcytic anemia of 3,250,000 red cells, 10.5 Gm of hemoglobin, 7,200 leukocytes with 52 per cent neutrophils, 10 per cent eosinophils, 2 per cent basophils, 33 per cent lymphocytes, 3 per cent monocytes, and an increased erythrocyte fragility range of 450 to 366. Thus, it would seem that the evidence for a maternal, familial inheritance of splenic instability is present here, and that in this patient not only was a hemolytic process precipitated spontaneously, but also a concomitant profound neutropenia and thrombocytopenia, all three of which were promptly and dramatically corrected by surgical removal of the spleen.

Figure 3 summarizes the hematologic data in five patients, four females and one male, ranging in age from 6 to 67 years, in whom removal of the spleen was followed by a prompt and sustained hemolytotoxic re-equilibration. The bone marrow in every instance showed marked generalized compensatory cellular hyperplasia of all normal cell strains, without invasive foreign cell or toxic manifestations.

SPLENIC PANHEMATOPENIA, SECONDARY TO GAUCHER'S DISEASE

The spleen is secondarily involved in a great many general, constitutional, pathologic states, and we have observed for some time the tendency under such circumstances for the R-E system of cells to exercise their physiologic functions erratically and to an exaggerated degree. In one patient, Mrs. C. P., for example, with proven Hodgkin's syndrome, in whom the spleen was primarily and predominantly involved, both an anemia and neutropenia, requiring weekly whole blood transfusions, were relieved only following splenectomy, and a clinical remission of two years ensued. In another recent patient, Mr. F. J., with known Hodgkin's disease, a thrombocytopenic purpura, refractory to all medical measures, developed, and splenectomy was elected as a last resort and was followed by a return of the platelets and the disappearance of all purpuric manifestations. Another patient, Mr. J. B. G., with characteristic thrombocytopenic purpura hemorrhagica, with an entirely typical megakaryocytic hyperplasia in the bone marrow, and with the spleen not palpable, received an immediate and complete clinical and hematologic remission following excision of the spleen, which when removed, however, was grossly enlarged and showed pathognomonic Hodgkin's lesions microscopically. While without the usual signs and symptoms, and unsuspected preoperatively, the subsequent course of events fully confirmed the histopathologic diagnosis from the spleen.

The most classical, clinical example in our experience, of the indiscriminate sequestration of all circulating cellular elements by a spleen, enlarged and engorged secondary to a generalized constitutional disease, is that of the following young woman with Gaucher's disease.

Case 3. E. S., a 20 year old, white, single female was admitted to the Hematologic Service, University Hospital, April 15, 1945, with the chief complaint of a large abdominal tumor. * She had first noted some discomfort in the left upper quadrant

* Referred by Dr. Roy Barnwell of Akron, Ohio, to whom we are deeply indebted for the family history and for cooperation in the study of the two sisters in this interesting family.

two years previously. Enlargement of the abdomen had been gradual and painless but steady. She had bruised easily from early childhood, resulting in a gradually increasing mottled pigmentation of both lower extremities. There were increasingly frequent episodes of epistaxis. Her menstrual periods had become established on a thirty days cycle at age 13, with marked menorrhagia regularly lasting seven days or longer. Slowly increasing pallor, associated with the development of exertional dyspnea and an accentuation of all hemorrhagic phenomena, finally led her to seek medical advice from the family physician.

It became evident upon questioning that her reluctance to seek medical attention derived from knowledge of a similar abdominal enlargement in a younger sister some years earlier, requiring surgical management, which this patient feared. The patient was one of three children, two girls and one boy, of maternal Swedish and paternal German-Swiss parentage. In 1933, the sister, who is two years younger, was taken to Dr. Barnwell at the age of 6 years for a large intra-abdominal tumor, which had been noted since infancy by the parents, and was associated with episodes of vomiting and epistaxis. Examination at that time revealed an enormous spleen filling almost the entire abdomen, with a moderate enlargement of the liver. The superficial veins of abdomen and chest were dilated and prominent. Serial laboratory studies during an eight day period of observation revealed a progressive leukopenia, 3,600 to 2,300 white cells with a normal differential, a falling red cell count, 4,200,000 with 13.9 Gm hemoglobin to 3,240,000, hemoglobin 10.5 Gm, and 74,000 platelets. The bleeding time was 9 min., the coagulation time $3\frac{1}{2}$ min. Banti's disease was the tentative diagnosis, and splenectomy was advised. A spleen

10 or 12 times normal size was successfully removed. Convalescence was uneventful, and on discharge three weeks postoperative the white cells were 11,000 with a normal differential, the red cells 4,300,000, hemoglobin 14.3 Gm, and platelets 320,000. Sections from the spleen were subsequently submitted to a number of pathologists, including Dr. E. L. Saylor, Akron, Dr. P. Morse, Detroit, Dr. H. R. Wahl, Kansas City, and to our group, all of whom concurred in the diagnosis of Gaucher's disease. Four months following splenectomy, this child was severely burned over the back from hair line to heels, requiring rehospitalization. Recovery was prompt and complete, and the blood reacted normally throughout the recovery period. In the intervening years, growth and development have been entirely normal, and no clinical symptoms or obvious signs of any constitutional disturbance have reappeared. The current hemogram as determined in this laboratory, at the time of the sister's admission, April 15, 1945, was as follows: WBC, 12,950, RBC, 4,110,000, hemoglobin, 12 Gm, reticulocytes, 1.4 per cent, platelets, 798,000. Supravital differential: PMN, 32 per cent, PMB, 1 per cent, PME, 6 per cent, lymphocytes, 53 per cent, monocytes, 8 per cent. Skeletal x-rays showed thinning of the cortex and expansion of the lower third of the femur and also cortical thinning of the tibiae, significant of Gaucher's disease. A sternal marrow study revealed a hyperplasia with moderate left shift in both erythroid and myeloid elements, with an increase in very young monocytes and monoblasts. A few scattered Gaucher cells were present.

No other member of this family has shown any evidence of familial disease.

On the basis of his preceding experience, a tentative clinical diagnosis of Gaucher's disease was made by Dr. Roy Barnwell, when the second sister consulted him. His initial laboratory data, April 2, 1945, showed WBC, 3,100, RBC, 3,270,000, hemoglobin, 10 Gm, and again on April 10 WBC, 2,900, RBC, 3,000,000, hgb, 9.2 Gm. On admission to the University Hospital, the physical examination showed a well developed, well nourished young woman with prominent abdomen, in no obvious distress at rest. Temperature, 98.6°, pulse, 82, respirations, 20, blood pressure systolic, 120, diastolic, 84 mm Hg. There was moderate pallor of skin, mucous membranes, and nail beds. Glasses corrected for astigmatism and hypermetropia, but there had been recurring conjunctivitis and the sclerae were injected and reading was uncomfortable. There were minimal medial pinguiculae bilaterally. On the left lower and upper eyelids were small papillomatous growths. Fundoscopic examination was negative. There was no generalized lymphadenopathy. The lungs were clear to percussion and auscultation, but the heart was displaced upward and to the left with an elevated left diaphragm. The spleen, somewhat tender to deep palpation, filled practically the entire abdomen, the right edge with notch extending 3 cm. to the right of the midline and downward to within 2 cm. of the symphysis pubis. Enlargement of the liver could not be detected. Extending from the patella to below the malleoli on both legs was a diffuse mottled, reddish brown pigmentation, without edema. Neurologic examination was entirely physiologic.

Admission blood studies showed only 650 total white cells, 3,130,000 red cells, 8 Gm. of hemoglobin, and 68,860 platelets (fig. 4). The differential white count, as nearly as it could be determined, gave 46 per cent mature neutrophils, 48 per cent normal lymphocytes, and 6 per cent monocytes, with normochromic, normocytic erythrocytes. Bleeding time was 6½ min., coagulation time 6 min. The Rumpel-Leede test was positive. Corrected erythrocyte sedimentation index was 0.1 mm. per min. Erythrocyte fragility range was 0.450 to 0.280. Sternal marrow aspiration yielded a grossly hyperplastic tissue with normoblasts predominating, and the following supravital differential cell count: Myeloid: erythroid ratio, 1:4.2, neutrophilic myelocytes C, 80 per cent, myelocytes B, 0.5 per cent, PME, 6.5 per cent, PMB, 0.5 per cent, Gaucher's cells, 1.0 per cent, megakaryocytes, 3.0 per cent, phagocytic clasmatoocytes, 8.0 per cent, plasma cells, 0.5 per cent. Erythroid elements: normoblasts, 96 per cent, erythroblasts, 2.7 per cent, megaloblasts, 1.3 per cent. Single, scattered, but rare Gaucher's cells served to establish the diagnosis, though no focal accumulations could be found. There was no significant left shift or diminution in the myeloid elements, though there was a moderate relative increase in eosinophils. There was some focal increase in highly phagocytic clasmatoocytes, none of which, however, contained abnormal fat vacuolization of Gaucher fibrils, and there was no increase or qualitative change in the monocytes. Only a rare plasma cell was observed, with qualitatively normal though slightly diminished megakaryocytes. Mitoses were rare in all cell strains. Other laboratory findings included normal urinalysis, an NPN of 7 mg. per cent, fasting blood glucose 95 mg. per cent, and negative serology. An adrenalin test gave transitory, significant increases in all of the circulating blood elements, as recorded in figure 4,

coincident with an appreciable shrinking of the spleen. X-ray examination of the long bones showed cortical thinning of the distal ends of both femurs and of the proximal ends of both tibiae suggestive of Gaucher's disease.

No contraindications having been discovered, and the particularly profound leukopenia persisting, splenectomy was urgently advised, and on April 19 a 5,100 Gm spleen, measuring $41 \times 20 \times 8.5$ cm, was removed by Dr. Verne Dodd without incident or surgical difficulty. The fresh organ was soft, pinkish red in color, and on cut surface many small pinpoint whitish areas were noted. On supravital examination of scrapings of the freshly cut surface, great syncytial-like sheets of large Gaucher cells were seen replacing much of the parenchyma. The cells were non-motile, varied from 30 to 50 micra in diameter, with a single eccentric nucleus, and 1 to 2 nucleoli, occasional cells contained as many as 4 nuclei. The cell membrane was delicate and easily ruptured. The cytoplasm was packed with Gaucher fibrils which were from 8 to 10 micra long, with fusiform tapered ends. They were seen as slightly to markedly curved bodies, grouped and arranged in strata-like formation around the nucleus. No mitochondria were seen. There was very little free cytoplasmic substance, owing to the compactness of the fibrils. The nuclei were 5 to 7 micra in diameter, and the chromatin was blotchy as described by Erf,¹² with nucleoli occasionally noted. The nuclear membrane was well defined. None of the spheroid cytoplasmic granules described by Erf were noted. Films of the splenic scrapings, made by diluting with human serum and stained with Wright's Giemsa stain, confirmed the delicate character of the easily ruptured cell membrane and the thick blotchiness of the nuclear chromatin. The cytoplasm in these fixed preparations appeared to be composed of fine, light-blue-staining, reticular strands, with none of the fibrillar outlines discernible.

Liver biopsy confirmed the normal gross appearance of this organ, and no lymph nodes could be found in the mesentery for histologic study.

On chemical analysis the spleen was found to contain 10.3 per cent lipids. Further lipid fractionation and biologic studies are being carried out and will be reported later.*

The postoperative course was uneventful, with prompt re-establishment of a normal, peripheral hematologic picture (fig. 4). The patient was discharged on the ninth postoperative day and was seen again one month later. Both clinically and hematologically, she has resumed a completely normal re-equilibration, with no complaints and an entirely changed psychology.

A survey of the literature, while recording the more or less striking influence of the splenomegaly on the circulating blood cells in Gaucher's disease, as proved by the hematopoietic re-equilibration which follows splenectomy, has failed to show an instance of so extreme a leukopenia with associated anemia and thrombocytopenia as the one cited here. It is a matter of degree only, however, and the inclusion of this case serves only to illustrate the general thesis relating to splenic dysfunction under pathologic conditions. Jaffe¹⁴ has reviewed in great detail the pathogenesis of Gaucher's disease. The younger the Gaucher cell the less distinct is the fibrillation of the cytoplasm, and it is between these fibrils that the specific storage of kera-

* We are indebted to Dr. George Scheff for these analyses.

and cerebrum,¹⁵ hydrophobic lipids, takes place, giving these cells their coarsely vacuolated appearance. It is possible that the great predominance of fibrils and the relative paucity of vacuolization of the Gaucher cells in our two patients reflect a generation of older, more mature cells and may correlate with the striking clinical chronicity and tissue localization of the pathognomonic cellular hyperplasia. The distinctive characteristics of these cells, as we have seen them in the supravital technic, have been adequately discussed and effectively illustrated by Erf.¹³

Differential diagnosis is at times difficult. Petit and Schleicher¹⁶ cite an instance in which sternal marrow aspiration established the diagnosis of Gaucher's disease in an unexplained atypical anemia in a Jewish male 79 years of age, and Reisman and Utz¹⁷ emphasize their failure to find Gaucher cells in the marrow of a 10 year old Jewish refugee girl, in whom subsequently splenic puncture was performed with confirmation of the diagnosis.

Wilensky¹⁸ has reviewed the indications for splenectomy with particular emphasis upon the otherwise uncontrollable hemorrhagic manifestations. Mandelbaum and Berger¹⁹ report the removal of a 6,822 gram spleen for thrombocytopenia and hemolytic anemia, secondary to Gaucher's disease, with lipid analyses by Sobel and Kaye.²⁰ Dameshek²¹ reports a case of Gaucher's disease and marked pancytopenia in which splenectomy was performed because of a well defined hemorrhagic tendency and because the spleen was so large that it interfered with the child's locomotion. Following splenectomy, there was a dramatic response in all the blood elements, which has been sustained for seven years. Naegeli²² attributed the changes in the circulating blood cell equilibrium to a hyperfunction of the spleen, and not to an infiltration of the hematopoietic organs by the Gaucher cells. Dameshek concurs with these observations.

DISCUSSION

Our studies fully confirm Naegeli's interpretation of splenic pathologic physiology, but we would not limit it to Gaucher's disease. The additional advantage of observing and analyzing fresh, surgically removed, splenic tissue in the supravital technic, which identifies both the phagocytic cells and their engulfed content, strongly incriminates the splenic macrophages in all of the co-called hypersplenic syndromes. Platelets, as well as leukocytes and erythrocytes, can be readily recognized when present within the living splenic phagocytes, and the proportion of each, thus discovered sequestered and being destroyed in any spleen, is generally a good reciprocal index of the cellular units probably available in the circulation, when the bone marrow is not depressed. The number of phagocytic cells in an enlarged spleen, weighing 2 to 6 kilograms and frequently showing 10 to 12 macrophages per oil immersion field of the microscope, assumes astronomical figures, and the destructive capacity is correspondingly enormous. The immediacy and magnitude of the increase in circulating elements following splenectomy is a measure of the tremendous bone marrow potential, revealed only by the elimination of an abnormal splenic influence.

The differential diagnoses, which must be considered objectively and judiciously

by the clinician in the syndrome we are here discussing, are threefold (1) a hypoplastic bone marrow, either primary, idiopathic, or secondary to some noxious agent,²³ (2) splenic panhematopenia secondary to some constitutional pathologic process which involves the spleen predominantly and disturbs its finely adjusted physiologic functional balance, and (3) primary splenic panhematopenia on a congenital or familial basis. Complete data from the adrenalin test* and careful sternal marrow aspiration analyses should provide the information upon which to base an opinion and advise therapy. Elective splenectomy in the first two mechanisms may, and frequently does, result in a more or less temporary and abortive but definite remission of those signs and symptoms dependent upon the disturbed cellular balance, and it may or may not influence materially the fundamental underlying disease, but in the last named syndrome, where the spleen apparently is primarily at fault, a prompt, complete, and permanent re-equilibration, hematologic and clinical, may be anticipated and predicted with some assurance.

SUMMARY

1 The spleen is an organ of multiple structures and many functions, but in the interests of human health and disease, it is probably far more important pathologically than physiologically.

2 It has been abundantly proved that instability in splenic functional balance toward any one of the essential elements of the blood passing through this organ may be an inherited trait, as in congenital hemolytic icterus. Recognition is now made of a syndrome in which, despite intensive compensatory panmyeloid hyperplasia, indiscriminate elimination of all circulating elements occurs, actually simulating panmyeloid hypoplasia. Splenectomy in such a syndrome is often dramatically curative. Primary splenic panhematopenia is suggested as an appropriate descriptive designation.

3 The potentially important role which may be played by the spleen, secondarily involved in a wide variety of syndromes, with the precipitation of varying degrees of peripheral cellular disequilibria, demands careful diagnostic discrimination. A dependable experience in the specific technics by which bone marrow and splenic functions are appraised is essential to sound judgment and clinical acumen.

4 The normal spleen is apparently not essential to life and health at any age and, therefore, may be surgically removed without prejudice to future hemolytopoietic equilibria and longevity. The pathologic spleen may at times constitute a very real hazard to health and an actual threat to survival, in the more acute syndromes, prompt surgical intervention may be lifesaving.

* Technic of the adrenalin test. During a fifteen to thirty minute base line period, under basal metabolic conditions, the pulse, blood pressure, and two preliminary complete peripheral blood studies are obtained and the splenic outline is traced. Depending upon the age and vascular integrity of the patient, 0.5 to 1 cc of 1:1000 adrenalin chloride is injected subcutaneously. Blood studies are repeated at ten minute intervals until the pulse and blood pressure reach their maximum stimulation, which usually coincides with the greatest contraction of the spleen. The peripheral blood studies are then continued at fifteen minute intervals until the spleen has relaxed and the biphasic depression of the curve has been obtained.

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THE MEGAKARYOCYTES IN IDIOPATHIC THROMBOCYTOPENIC PURPURA, A FORM OF HYPERSPLENISM

B₃ WILLIAM DAMESHEK, M D , AND CAPTAIN EDWARD B MILLER, A U S

IN his book *Opera omnia*, published in 1775, Paul Gottlieb Werlhof¹ devoted a chapter to "Morbus maculosus haemorrhagicus, which he had first described forty years previously. He wrote

An adult girl, robust without manifest cause, was attacked recently, towards the period of her menses, with a sudden severe hemorrhage from the nose, with bright but foul blood escaping together with a bloody vomiting of a very thick extremely black blood. Immediately there appeared about the neck and on the arms, spots partly black partly violaceous or purple, such as are often seen in malignant smallpox, moreover the number of the spots increasing and surrounding completely both of the eyes, the back of the nose and the skin around the mouth and chin with a livid black color like marked from bruises.

Since the bleeding began simultaneously with the menses and since there was spontaneous recovery, it is indeed probable, as most authorities have agreed since, that this was an example of idiopathic thrombocytopenic purpura. The reasons for the development of sudden, generalized bleeding from all the mucous membranes and into the skin are almost as obscure today as they were in Werlhof's time. In the present paper, an attempt is made to develop a concept of pathogenesis centering about the failure of platelet growth from the megakaryocytes in the bone marrow, and dependent upon an abnormal inhibitory factor in a distant organ, namely, the spleen.

The great diminution in platelets in Werlhof's disease was first recognized by Krauss² in 1883 and by Denys³ in 1887. Hayem⁴ later confirmed and amplified these isolated observations. The relationship of the platelets to the giant cells of the bone marrow—the megakaryocytes—became known with the work of J. H. Wright⁵ in 1906 and 1910. In 1915, Frank⁶ made accurate studies of essential thrombopenia and postulated a marked diminution in platelet production by the megakaryocytes. * In the following year, Kaznelson⁷ suggested splenectomy as a therapeutic maneuver in a chronic relapsing case of the disease. He assumed, by analogy with hemolytic anemia, that the spleen might have an unusual thrombolytic function. The results of the first operation were brilliant, but in the next two cases⁸ only temporary increases in platelets occurred. Since that time the favorable effect of splenectomy in idiopathic thrombocytopenic purpura has been amply confirmed. The quick recovery following splenectomy of many desperately ill patients bleeding spontaneously from all the orifices is one of the most dramatic events in medicine, and must immediately implicate the spleen as of prime pathogenetic importance in the disease. In confirmation of this, the injection of splenic

From the Blood Laboratory of the Boston Dispensary and the J. H. Pratt Diagnostic Hospital, aided by grants from the Charlton Fund, Tufts College Medical School, and the Upjohn Company.

* Frank is incorrectly quoted by most observers as having suggested a splenic effect on megakaryocyte platelet growth.

extracts from patients with the disease has, in the hands of several investigators, resulted in the development of thrombocytopenia in the experimental animal.

Whether the diminution in platelets is due to increased thrombolysis by an abnormally active spleen or to diminished platelet production by the megakaryocytes has remained a question to the present time. Although Frank's studies^{6,16} of the bone marrow demonstrated a definite disorder of platelet production by the megakaryocytes, the significance of his observations has not been fully appreciated and most observers simply conclude that the spleen destroys platelets excessively. The present paper deals with a study of the megakaryocytes in bone marrow biopsies from typical cases of the disease, and from symptomatic cases in association with splenomegaly, together with their comparison with normal controls. Serial studies of the bone marrow before and after splenectomy have demonstrated the remarkable effects on platelet production which occur shortly after operation. Our findings are in accord with Frank's concepts that the fundamental defect leading to thrombocytopenia is a dysfunction of the megakaryocytes of the marrow. In turn, this appears to be due to a form of abnormal splenic activity, i.e., one of the types of hypersplenism, and the disease may thus be considered fundamentally as a disorder of the spleen, with the bone marrow and the blood being secondarily involved.

MATERIAL

The material studied included the following:

1. Ten normal cases, all with hemoglobin values above 12.4 Gm. per 100 cc. and red cell counts above 4.0 M. per cu. mm. The platelet levels (Dameshek method) were within normal range of 400,000 to 900,000 per cu. mm.

2. Eleven cases of idiopathic thrombocytopenic purpura. Of these, 5 were acute cases in which splenectomy was performed. The usual criteria for the diagnosis of this condition were present: namely, spontaneous bleeding into the skin and from mucous membranes, low platelet count, prolonged bleeding time, normal coagulation time, poor retraction of clot, positive tourniquet test, absence of anemia other than that explainable on the degree of blood loss, and no evidence at bone marrow biopsy of leukemia or other fundamental hematopoietic disease. Bone marrow punctures were performed prior to splenectomy, and at varying intervals after splenectomy. In 6 chronic cases, single bone marrow punctures were performed.

3. Five cases of thrombocytopenia associated with well defined splenomegaly of nonleukemic and non-neoplastic origin (symptomatic hypersplenic thrombocytopenia), as follows: Gaucher's disease, unknown origin, probably infectious, juvenile hepatic cirrhosis, probable splenic vein thrombosis, and Felty's syndrome.

4. A miscellaneous group of hematologic conditions, in which systematic counts of the megakaryocytes were not made but in which a general impression regarding the number and condition of the megakaryocytes in the bone marrow was noted. This group included about 50 cases of idiopathic thrombocytopenic purpura, a similar number with thrombocytopenia which was symptomatic of an underlying splenic condition, about 300 cases of leukemia of various types, and

several hundred cases of various types of anemia, polycythemia, and other blood dyscrasias

METHODS

Peripheral Platelet Counts—The indirect (wet smear) technic of Dameshek,¹⁷ using an isotonic aqueous solution of sodium citrate containing brilliant cresyl blue, was used. The normal platelet count by this method ranges from 400,000 to 900,000 per cu. mm., with an average normal count of about 600,000 per cu. mm.

Sternal Puncture—This was performed by the introduction of a simple sternal puncture needle through the anterior lamella and into the marrow space, at a point on the sternum between the third and fourth intercostal spaces. After withdrawal of the stilet, a very small amount of marrow fluid, usually 0.3 cc. or less, was withdrawn by aspiration with a dry 5 or 10 cc. syringe. Drops of aspirated material were immediately placed, without the further use of anticoagulant or other material, on carefully cleaned new glass slides and gently spread with minimum pressure by means of another slide. The preparations were allowed to dry in air and stained first with Wright's stain, then with Giemsa stain, following which coverslips mounted in balsam were affixed.

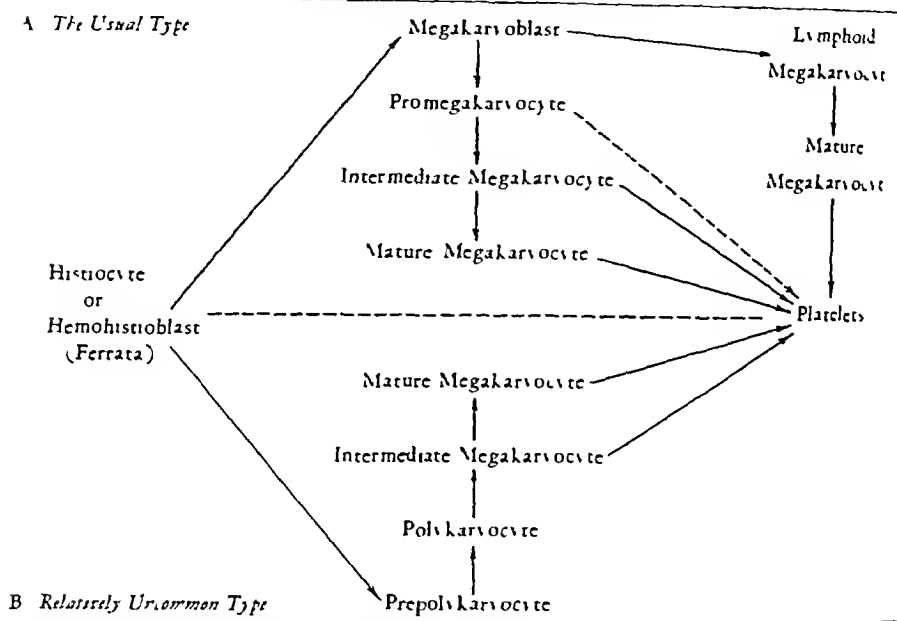
Megakaryocyte Counts—A rectangle 20 x 15 mm. was cut out of paper and placed over the slide to be studied. The megakaryocytes in this area, containing approximately 20,000 oil immersion fields, were counted and expressed in terms of a million nucleated cells. At least one-half million nucleated cells were counted, the count being facilitated by first accurately enumerating the nucleated cells in 20 oil immersion fields.

Differential Counts of the Megakaryocytes—The identification of megakaryocytes can be either very easy or quite difficult. The typical huge adult forms are readily defined, but recognition of their precursors requires much patience and study. In studying the megakaryocytes the work and nomenclature of various Italian investigators, notably Di Guglielmo,¹⁸ Morone,¹⁹ and Torrioli and Scalfi,²⁰ have been followed with slight modifications.

The predominant mode of origin of the megakaryocyte is probably from a stem cell or megakaryoblast, which in turn probably originates from the pluripotential histiocyte or hemohistioblast (table 1). A subsidiary method of origin, about which much controversy has taken place, is that from the polykaryocyte or osteoclast. Although this cell was for many years sharply differentiated from the megakaryocyte, its close relationship to the latter was demonstrated by several Italian investigators. Di Guglielmo¹⁸ concluded that the polykaryocyte was derived from the fusion of primitive mononuclear histioid cells, with the resultant development of large multinucleated giant cells, which in turn became megakaryocytes. Di Guglielmo's observations were confirmed by Bianchini,²¹ Cesa-Bianchi,²² Morone,¹⁹ Fontana,²³ and Gandolfo,²⁴ and in this country by Rosenthal.²⁵ Morone¹⁹ sharply differentiated the polykaryocytes from osteoclasts, but in this he was disputed by Lambin and Lamers.²⁶ On the other hand, the origin of megakaryocytes from prepolykaryocytes was rejected by Lapidari²⁷ and by Wujts.²⁸

Other more uncommon methods of megakaryocytic development have been cited

by various investigators. Thus Bloom²⁹ described them as originating from nonphagocytic fixed tissue cells lining the sinusoids of the liver. Downey, Palmer, and Powell,³⁰ by studying a case of atypical myelosis, traced the origin of megakaryocytes from reticulum and myeloblasts. Later, Downey and Nordland,³¹ in studying a similar case, found transitions from myeloblasts to platelet-producing megakaryoblasts in the peripheral blood, and transitions from hemocytoblasts to megakaryocytes in the spleen. No formation of megakaryoblasts from the splenic reticulum was apparent.

TABLE 1—*Stages of Megakaryocyte Formation*

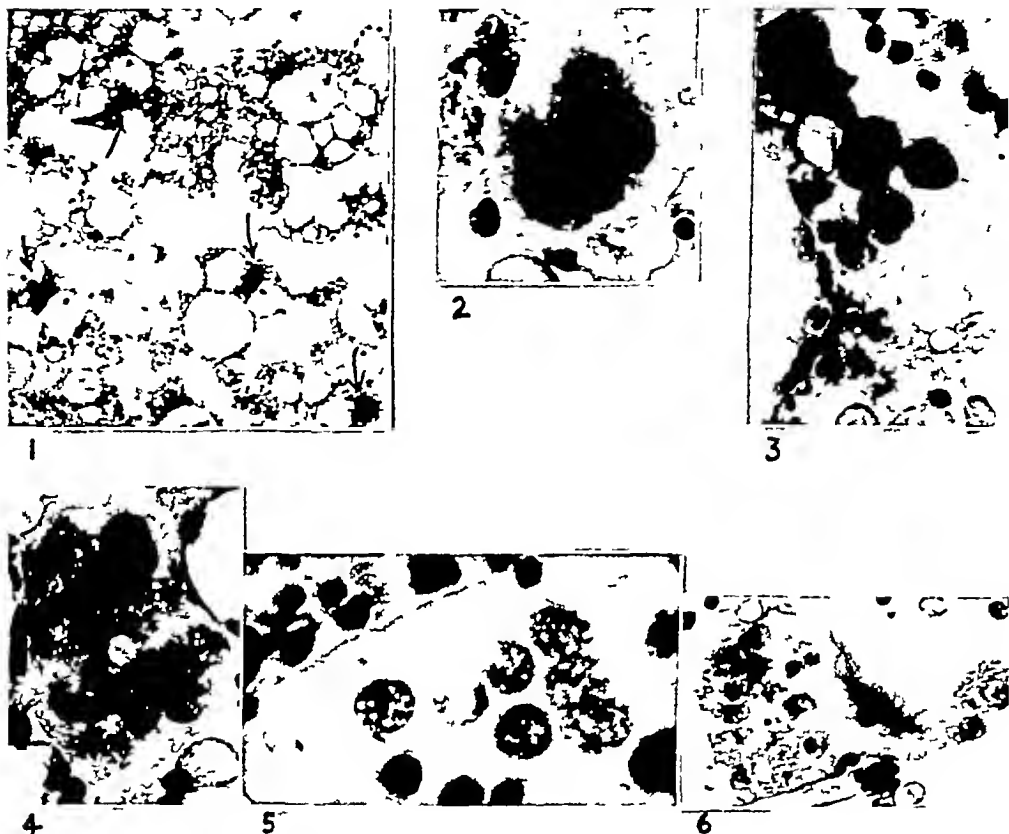
Megakaryocyte formation has at least two methods of derivation (A) the usual, by way of megakaryoblast and (B) the relatively uncommon from a prepolikaryocyte. Both of these stem cells appear to be derived from the pluripotential histiocyte or hemohistioblast of Ferrata. Platelet production occurs from the relatively mature types of megakaryocytes and to some extent from the promegakaryocytes.

The following megakaryocytes have been distinguished (see colored plate and photomicrographs)

Megakaryoblast These are cells about twice the size of myeloblasts with a blue nongranular cytoplasm and a large somewhat irregular single nucleus, which at times is kidney shaped, with numerous nucleoli. These cells do not produce granular or nongranular platelets either normally or in cases of idiopathic thrombocytopenic purpura. In normal preparations they comprise less than 1 per cent of all the megakaryocytes.

Promegakaryocyte Usually about the same size as the megakaryoblast, although occasionally much larger, with scant dark blue cytoplasm and a dense nonlob-

lated or partly lobulated nucleus with heavy chromatin. This form frequently has platelet-like bodies at the periphery of its cytoplasm and rarely nongranular cytoplasmic processes. When both these details are present, the cell is designated as one with granular platelet production. Cells with nongranular processes were



FIGS 1-6 NORMAL MARROW

FIGS 1 Sternal bone marrow puncture smear ($\times 75$). Note islands of cells with numerous fat cells, islands of marrow cells, and four megakaryocytes marked by arrows.

2 Sternal puncture smear ($\times 750$). Mature megakaryocyte. Note the coarse granularity of the cytoplasm with well defined platelet development at the edges, particularly in certain of the pseudopods.

3 4 Mature megakaryocytes ($\times 1000$).

5 Sternal puncture smear ($\times 1000$). Polynuclear cell, a multinucleated giant cell, an occasional forerunner of the mature megakaryocyte.

6 Sternal puncture smear ($\times 500$). Polynuclear cell with coarse granularity and platelet production.

absent or rare in normal cases but were frequent in the cases of purpura. Normally, promegakaryocytes make up about one third of the total number of megakaryocytes.

Lymphoid megakaryocyte. A large cell with basophilic cytoplasm, usually without

granules, and a nucleus which is relatively small in comparison with the entire cell and usually distinctly lobulated

Intermediate forms These cells are intermediate in size or intermediate in maturation between the relatively small promegakaryocyte and the huge adult forms. The cytoplasm is very heavily granulated. Platelet formation may or may not be present.

Adult megakaryocyte A cell of variable size, but usually very large, containing a single large multilobed nucleus with increasing density as it matures. The cytoplasm varies in color from blue to pink and contains a variable number of characteristic azurophilic granules grouped at first in the perinuclear zone. Typical platelets which are almost always granular are frequently found in pseudopod-like structures grouped in masses about the periphery of the cell.

Prepolykaryocyte A mononuclear cell which tends to occur in clusters. There is an abundant vacuolated blue cytoplasm without granules and a clear reticulated eccentric round nucleus with one or more nucleoli. The cells are seen normally and do not appear to be increased in purpura.

Polykaryocytes These are probably fused syncytia of the above—that is, a large number of individual nuclei lie within one cytoplasmic body. These cells are probably identical with the osteoclasts.

Adult megakaryocyte This cell is probably formed by nuclear fusion from the polykaryocyte.

Degenerated forms In these cells the cytoplasm is either homogeneous and hyaline in appearance, or there is marked vacuolization of the cytoplasm, or the nucleus is hyperlobulated and its cytoplasm nongranular.

It must be recognized that the relationships of the various types of cells to each other may be more artificial than real, since they are based on a study of what appear to be transition forms. Because of this uncertainty, the megakaryoblasts and promegakaryocytes may be designated as young forms, and the lymphoid megakaryocytes, intermediate forms, and adult types as "adult forms," with a separate designation for the degenerated cells.

OBSERVATIONS

A. *Normal Cases* (table 2).—In 10 normal patients with normal hematologic findings, study of the megakaryocytes revealed the following features:

1. Not more than 300 megakaryocytes per million nucleated cells were present.
2. About two thirds of the megakaryocytes contained platelets or platelet-like bodies at the peripheries of their cytoplasm.
3. Megakaryoblasts were rare.
4. Promegakaryocytes, usually producing granular platelets, were plentiful. Nongranular platelet production was rare. Approximately one half of the platelet-producing megakaryocytes were young forms.
5. Degenerated forms varied from 8 to 22 per cent of all cells.

B. *Acute Idiopathic Thrombocytopenic Purpura* (table 3).—Differential counts of the megakaryocytes were made in 5 cases of acute idiopathic thrombocytopenic purpura before splenectomy. In 4 of these, one or more marrow studies were made after splenectomy. The findings before splenectomy were as follows:

TABLE 2.—Normal Controls—Differential Megakaryocyte Counts

Name	R B C (M per cu mm)	Platelets (Thousands per cu mm)	Blasts	Promegakaryocytes			Lymphoid megakaryocytes		Intermediate megakaryocytes		Adult megakaryocytes		Degenerated forms				Mitotic figures	Megakaryocytes per million nuc. cells	Megakaryocytes (% platelets)
				± plts	± gran plts	± nongran plts	± plts	± plts	± plts	± plts	± plts	± plts	Eos	Vacuol	Hyal	Loss of gran			
H P	4 39	480	0	6	13	1	0	0	9	0	45	13	4	3	0	6	2	190 9	68
E DeM	4 97	744	0	3	35	0	3	5	8	9	14	15	0	2	0	6	0	99 9	60
A F	4 96	420	0	2	42	0	0	1	12	0	32	2	0	3	0	6	2	266 8	86
A E	4 97	548	0	5	35	0	1	0	10	2	23	12	0	4	0	8	1	266 9	69
A McL	5 17	537	0	1	27	0	1	0	10	2	35	5	14	1	0	4	0	170 3	73
J B	3 94	484	2	0	32	0	2	2	4	0	14	32	0	0	0	12	0	191 3	52
C C	4 85	632	1	6	42	0	7	2	7	0	20	3	0	2	0	10	0	109 6	76
J W	4 70	410	2	2	38	0	2	6	8	9	24	8	2	2	0	6	0	129 6	72
A C	4 43	574	0	0	28	2	2	6	8	4	12	16	2	4	0	16	0	190 8	50
E D	4 46	594	0	0	26	0	4	0	18	4	32	4	2	0	0	10	0	209 3	80
Average	4 68	542	0 5	2 5	31 8	0 3	2 2	2 2	9 3	2 1	25 1	11 0	2 4	2 4	0	8 4	0 5	182 4	68 6
Range	3 94-51 7	420-744	0-2	0-6	13-42	0-2	0-7	0-6	7-18	0-9	12-45	2-30	0-14	0-4	0	4-16	0-2	99-270	50-86

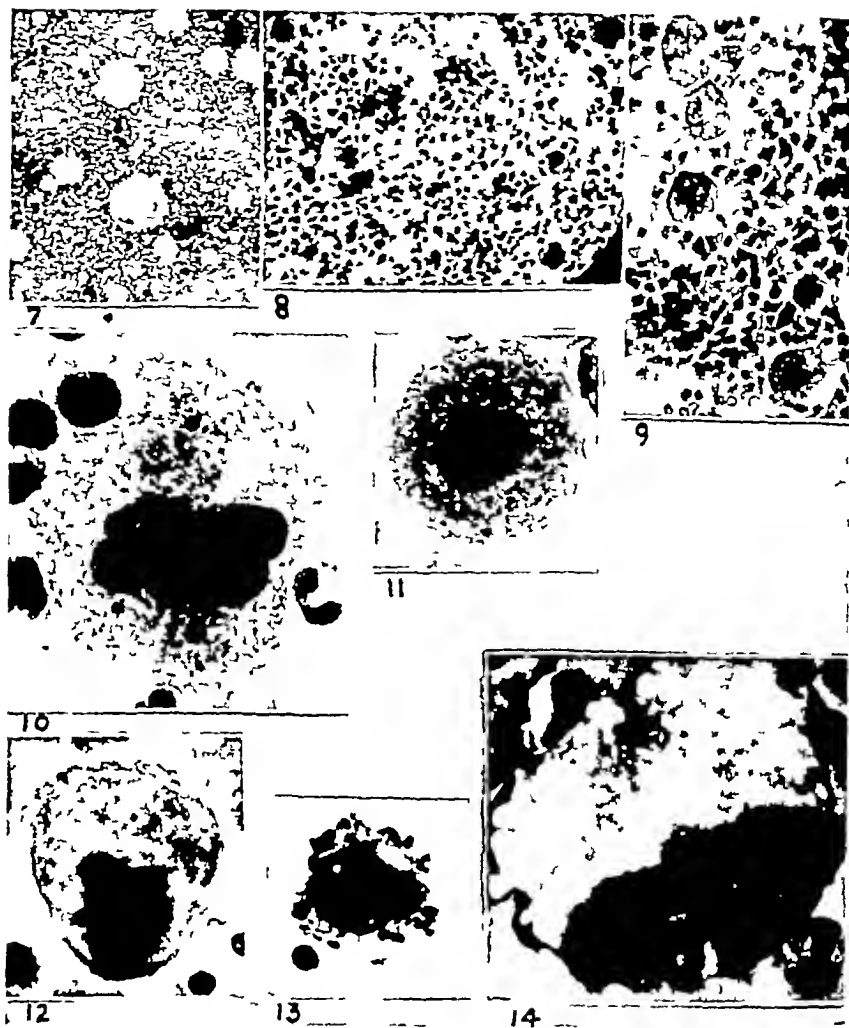
KEY TO ABBREVIATIONS IN TABLES

M = Millions
 R B C = Red blood cell count
 Platelets = Blood platelet count thousands per cu mm
 Plts = Platelets
 Gran = Granular or granularity
 Nongran = Nongranular
 Megs = Megakaryocytes

Eos = Eosinophilic
 Vacuol = Vacuolated
 Hyal = Hyaline
 Nuc = Nucleated
 Blasts = Megakaryoblasts
 Promegas = Promegakaryocytes
 Intermed = Intermediate forms

TABLE 3.—Acute Idiopathic Thrombocytopenic Purpura—Differential Megakaryocyte Counts

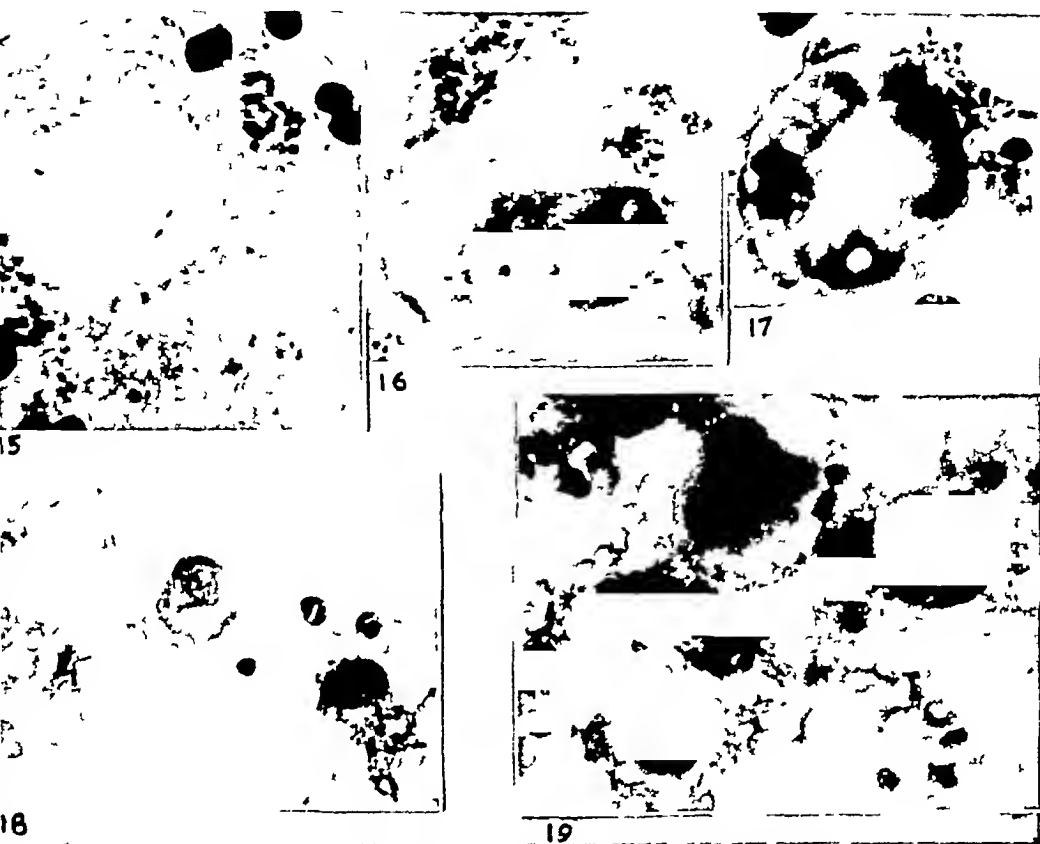
Name	Promegakaryocytes				Lymphoid megakaryocytes		Intermediate		Adult		Degenerated forms				Mitotic figures	Megakaryocytes per million nuc. cells	Megakaryocytes (% platelets)
	Blasts	± plts	± gran plts	± nongran plts	± plts	± plts	± plts	± plts	± plts	± plts	Eos	Vacuol	Hyal	Loss gran			
A K	2 9	17	7	1	7	0	5	0	35	2	1	0	14	1	1	546 9	18
L Z	1 5	9	6	1	9	0	0	0	45	3	3	0	9	0	1	501 3	19
I G	2 9	1	3	3	7	0	9	5	50	0	2	0	9	2	2	743 9	9
R L	8 8	9	13	3	14	5	4	1	14	1	3	0	17	1	1	366 4	18
M D	1 9	0	2	2	6	0	7	6	59	0	0	0	8	0	0	460 0	8
Average	2 88 0	7 2	6 2	2 0	8 6	1 0	5 0	4 2	40 6	1 2	1 8	0	11 4	0 8	0 8	523 7	14 4
Range	1-8	5-9	0-17	1-13	1-3	6-14	0-5	0-9	0-9	14-50	0-3	0-3	0	8-17	0-2	366-743	8-19
Normal	0-2	0-6	13-42	0-2	0-7	0-6	7-18	0-9	12-45	2-32	0-14	0-4	0	4-16	0-2	99-270	50-86



FIGS 7-14 IDIOPATHIC THROMBOCYTOPENIC PURPURA

- FIGS 7 Sternal puncture smear ($\times 65$) Large numbers of megakaryocytes about 25 in one low power field and about 5 to 8 times the normal number
- 8 Sternal trephine biopsy section ($\times 350$) Increase in megakaryocytes which show diminished granularity of cytoplasm and lack of platelet production
- 9 Sternal trephine biopsy section ($\times 500$) Mature megakaryocytes with conspicuous lack of granularity and complete absence of platelet production
- 10 Sternal puncture smear ($\times 1500$) Mature megakaryocyte Well defined granularity of cytoplasm rare platelet bodies no definite platelet formation
- 11 Sternal puncture smear ($\times 1000$) Mature megakaryocyte Well defined granularity no platelet formation sharp cytoplasmic edges somewhat pyknotic nucleus
- 12 Sternal puncture smear ($\times 1000$) Adult megakaryocyte Granularity of cytoplasm beginning degeneration at one end of cell No platelet production Beginning pyknosis of nucleus
- 13 Sternal puncture smear ($\times 1000$) Promegakaryocyte with formation of large bizarre platelets
- 14 Sternal puncture smear ($\times 1500$) Adult megakaryocyte no platelet formation marked degeneration of cytoplasm

1 The number of megakaryocytes per million nucleated blood cells was on the average about three times greater than in the normal



FIGS 15-19 IDIOPATHIC THROMBOCYTOPENIC PURPURA, POSTSPLENECTOMY

FIGS 15, 16 Twenty four hours after splenectomy Sternal puncture smear ($\times 1500$) Mature megakaryocyte Well defined beginning platelet production at edges of cell

17 Twenty-four hours after splenectomy Sternal puncture smear ($\times 1500$) Mature partially degenerated megakaryocyte with active platelet formation at one edge

18 Twenty four hours after splenectomy Sternal puncture smear ($\times 750$) Promegakaryocyte (right) with active granular platelet formation and intermediate form (left) with beginning platelet formation

19 Forty-eight hours after splenectomy Sternal puncture smear ($\times 1000$) Intense platelet production with the production of masses of new platelets

2 Only 14.4 per cent of the megakaryocytes showed obvious platelet production, as contrasted with the normal cases in which approximately two thirds produced platelets

3 Megakaryoblasts were definitely increased, but the proportion of promegakaryocytes was decreased

4 Adult megakaryocytes produced fewer platelets than in normal or chronic cases. Most of the platelet production appeared to derive from promegakaryocytes

and was often abnormal in type, with the production of large nongranulated platelets

5 Degenerated and mitotic forms were not increased

In the 4 cases which were studied both before and after splenectomy, the megakaryocytes per million nucleated red cells remained at about the original level or became somewhat increased. However, following operation, platelet production became sharply increased, from the average of 16 per cent before splenectomy to 73 per cent. The huge masses of platelets proceeding from previously unproductive megakaryocytes, and which often occupied large parts of the microscopic field, offered a striking contrast to the findings before splenectomy.

C *Chronic Idiopathic Thrombocytopenic Purpura*—In 6 cases of thrombocytopenic purpura of mild to moderate severity and often present for many years, the following were noted

1 The number of megakaryocytes per million nucleated blood cells ranged from 450 to 1565—far above the normal average of 183

2 A third or less of the megakaryocytes showed evidence of platelet production

3 Megakaryoblasts were increased

4 Promegakaryocytes were much less plentiful than in the normal, but non-granular platelet production was frequently seen

5 There was very little platelet production from adult megakaryocytes

6 Degenerated forms were slightly increased over normal

D *Symptomatic Hypersplenic Thrombopenic Purpura* (table 5)—The megakaryocytes were studied in 5 cases in which persistent thrombocytopenia and leukopenia were associated with well defined splenomegaly. The cases were as follows: (1) Gaucher's disease, (2) unknown origin, probably infectious, (3) juvenile hepatic cirrhosis, (4) probable splenic vein thrombosis, (5) Felty's syndrome (rheumatoid arthritis with leukopenia). It was believed that these cases might be considered examples of a form of simple hypersplenism, i.e., an increase in the normal splenic function.

The findings were as follows:

1 An increase in megakaryocytes, in about the same proportion as that seen in the acute essential cases

2 A normal proportion of platelet-producing cells

3 No increase in megakaryoblasts or decrease in promegakaryocytes

4 Normal production of platelets from adult megakaryocytes

5 No increase in degenerated forms

E *Miscellaneous Cases*—A general impression of the number and type of megakaryocytes in the sternal marrow as obtained both by the puncture and trephine methods has been recorded in most of the hematologic cases studied in our laboratory since 1928. Except in poorly spread preparations, containing thick clumps of marrow tissue, and in which megakaryocytes might be numerous, the average number of megakaryocytes per low power microscopic field was just over 1, the range being from 0 to 5. Platelet formation at the edges of the megakaryocytes was visible in at least one half of the cells observed.

Anemias In the deficiency syndromes, notably pernicious anemia, megakaryo-

cytes were usually diminished, at times markedly so. In anemia due to actual disease or involvement of the bone marrow, including aplasia, leukemia, lymphosarcoma, etc., the megakaryocytes were conspicuously reduced. This was particularly true in acute leukemia, in which the almost total lack of megakaryocytes contrasted sharply with the presence of extreme cellular hyperplasia. At times

TABLE 4—*Chronic Idiopathic Thrombocytopenic Purpura—Differential Megakaryocyte Counts*

Name	RBC	Platelets	Blasts	Promegakaryocytes			Lymphoid megakaryocytes		Intermediate		Adult		Degenerated forms				Mitotic figures	Megakaryocytes per million nuc cells	E platelets (%)
				3 platelets	4 gran platelets	5 nongran platelets	6 platelets	7 platelets	8 platelets	9 platelets	10 platelets	11 platelets	Eosin	Vacuol	Hyal	Loss gran			
H H	4 23	143 800	3	9	5	7	4	6	2	5	3	41	1	0	0	14	0	788 1	14
R J	5 01	190 000	0	3	15	3	2	4	1	5	6	48	2	0	0	11	0	1564 9	24
S R	4 40	184 400	2	4	13	1	5	2	3	1	7	45	1	3	0	13	0	596 5	28
A D	5 13	112 900	0	4	1	0	0	5	0	3	9	56	0	1	0	21	0	451 2	10
A M	4 54	90 800	2	1	7	1	6	7	5	3	16	38	0	2	0	12	0	782 0	34
R P	4 14	204 000	3	9	6	8	4	15	3	10	14	26	1	0	0	1	0	516 7	27
Average		156 000	1.4	4.2	8.2	2.4	3.4	4.8	2.2	3.4	8.2	45.6	0.8	1.2	0	14.2	0	836.5	22
Range		90-184	0-3	1-9	1-15	0-8	0-6	2-15	0-5	1-10	3-16	26-56	0-2	0-3	0	1-21	0	451-1565	10-34
Normal range		410-744	0-2	0-6	13-42	0-2	0-7	0-6	7-18	0-9	12-45	2-32	0-14	0-4	0	4-16	0-2	99-270	50-86

TABLE 5—*Symptomatic Hypersplenic Thrombocytopenic Purpura—Differential Megakaryocyte Counts*

Name	B1 s	Promegakaryocytes			Lymphoid megakaryocytes		Intermediate		Adult		Degenerated forms				Mitotic figures	Megakaryocytes per m nuc cells	E platelets (%)	Blood platelets per c c m
		3 platelets	4 gran platelets	5 nongran platelets	6 platelets	7 platelets	8 platelets	9 platelets	10 platelets	11 platelets	Eosin	Vacuol	Hyal	Loss gran				
J S	1	5	24	0	16	4	0	0	37	8	0	0	0	5	2	462 0	67	133 600
V G	1	3	31	2	6	4	9	0	24	15	0	0	0	5	1	885 2	72	271 300
E P	0	2	41	3	9	9	1	0	13	11	1	1	0	9	3	257 8	67	149 600
S W	1	6	9	0	4	5	2	0	31	37	0	0	0	5	1	482 8	52	70 100
U S	2	6	12	3	11	11	10	2	15	21	0	1	0	6	0	381 7	51	255 000
Average	1 0	4.4	23.4	1.6	9.2	6.6	4.4	0.4	24.0	18.4	0.2	0.4	0.0	6.0	1.4	493.9	61.8	180 000
Range	0-2	2-6	9-41	0-3	4-16	4-11	0-10	0-2	15-31	8-37	0-1	0-1	0	5-9	0-3	258-885	51-72	71-271 000

this finding was of considerable diagnostic value, particularly in the differential diagnosis of thrombocytopenic purpura. In anemia due to increased blood loss, whether by hemorrhage or increased hemolysis, the megakaryocytes were usually conspicuously increased in number, with abundant platelet formation being visible from their cytoplasm.

Polycythemia In polycythemia vera, one of the most outstanding features of the

bone marrow was the great megakaryocytic hyperplasia, which in certain cases seemed to dominate the entire marrow picture. In secondary polycythemia, the megakaryocytes appeared to be normal in number.

Leukemias The striking diminution and even complete lack of the megakaryocyte in acute leukemia has already been mentioned. In chronic myelogenous leukemia, these giant cells were often greatly increased, particularly in the earlier stages of the disease. In chronic lymphatic leukemia, there was a gradual reduction in the number of these cells as the marrow became progressively infiltrated with lymphocytes.

Hemorrhagic diseases No particular changes, either quantitative or qualitative in type, were observed in hemophilia, vascular types of purpura, or in other less well defined hemorrhagic conditions.

DISCUSSION

A. Origin of the Blood Platelets—Idiopathic or essential thrombocytopenic purpura is primarily a disorder in which the blood platelets are conspicuously reduced, as a result of which blood escapes from capillaries with ensuing purpura and other hemorrhagic manifestations. Knowledge regarding the origin of the blood platelets is comparatively recent, dating from the writings of James Homer Wright⁶ in 1906 and 1910. Howell²² had previously (in 1890) named the giant cells of the bone marrow megakaryocytes to distinguish them from the multinucleated giant cells apparently related more closely to the bone and which were called polykaryocytes or osteoclasts. In 1906, Wright^{2a} for the first time described the origin of the blood platelets from the megakaryocytes of the bone marrow. By the use of his special eosin-methylene blue stain (now called Wright's stain) he could clearly recognize the platelets in stained fixed tissue and differentiate them from other histologic elements. In 1910, he^{2b} described his studies of the bone marrow of the cat, mouse, rabbit, guinea pig, white mouse, opossum, and man with particular reference to the megakaryocytes and the platelets. He found that the giant cells of the marrow often contained granules which were most numerous in the pseudopodial processes of cytoplasm projecting into the sinusoids of the marrow. In some megakaryocytes or their pseudopods, one or more small groups of granules were separated by a zone of hyaline cytoplasm from the rest of the cell. These masses of granules with their intervening cytoplasm had the identical staining reactions of platelets. He furthermore found that bodies identical in appearance with blood platelets were often found near pseudopods and that detached pseudopods were at times seen in blood channels. He therefore concluded that megakaryocytes produced platelets by direct budding from pseudopods which had entered the circulation of the marrow. Bunting²³ in 1909 confirmed Wright's findings in the rabbit and furthermore showed that an increase in megakaryocytes induced by bleeding and the use of turpentine and saponin was associated with an increase in platelets. He also found that both the granules of the megakaryocytes and the platelets give identical staining reactions with the supravital dyes brilliant cresyl blue and neutral red. Smith, Robinson, and Tyson²⁴ showed that the oxidase reactions of the granules of both the megakaryocytes and platelets were negative.

Further evidence substantiating the origin of platelets from megakaryocytes has been gained from experimental studies in which the number of megakaryocytes and the fragments issuing from their pseudopods was correlated with the number of blood platelets as modified by repeated bleeding (Bunting³⁵), the action of benzol (Weiskotten, Wyatt, and Gibbs³⁶), the use of antiplatelet serum (Bedson and Johnston³⁷), the use of saponin (Firket and Campos³⁸), and by blocking of the reticulo-endothelial system (Volterra³⁹). Injection of colloidal materials, notably india ink, in mice was followed by thrombocytosis, the appearance of megakaryocytes in the peripheral blood, and a simultaneous increase in their number in the spleen and bone marrow. This so-called blocking of the reticulo-endothelial system in inducing thrombocytosis may be unimportant as compared

TABLE 6—Summary of Differential Megakaryocyte Counts

Disease	Blasts	Promegakaryocytes			Lymphoid megakaryocytes		Intermediate		Adult		Degenerated forms				Mitotic figures	Megakaryocytes per mmuc cells	Percentage with plts
		q plts	c gran plts	c nongran plts	c plts	s plts	c plts	s plts	c plts	s plts	basin	Vacuol	Hyal	Loss gran			
Normals																	
Average	0.5	2.5	31.8	0.3	2.2	2.2	9.3	2.1	25.1	11.0	2.4	2.1	0	8.4	0.5	182.5	68.6
Range	0-2	0-6	13-42	0-2	0-7	0-6	7-18	0-9	12-45	2-32	0-14	0-4	0	4-16	0-2	99-270	50-86
Acute idiopathic thrombocytopenic purpura																	
Average	2.8	8.0	7.2	6.2	2.0	8.6	1.0	5.0	4.2	40.6	1.2	1.8	0	11.4	0.8	523.7	14.4
Range	1-8	5-9	0-17	2-13	1-3	1-14	0-5	0-9	0-9	14-50	0-3	0-3	0	8-17	0-2	366-743	8-19
Chronic idiopathic thrombocytopenic purpura																	
Average	1.4	4.2	8.2	2.4	3.4	4.8	2.2	3.4	8.2	45.6	0.8	1.2	0	14.2	0	836.5	22
Range	0-3	1-9	1-15	0-8	0-6	2-15	0-5	1-10	3-16	26-56	0-2	0-3	0	1-21	0	450-1565	10-34
Symptomatic hypersplenic purpura																	
Average	1.0	4.4	23.4	1.6	9.2	6.6	4.4	0.4	24.0	18.4	0.2	0.4	0.0	6.0	1.4	493.9	61.8
Range	0-2	2-6	9-41	0-3	4-16	4-11	0-10	0-2	15-31	8-3	0-1	0-1	0	5-9	0-3	258-885	51-72

with the actual effects of the colloidal particles on the platelets themselves. One of us⁴⁰ showed in unpublished experiments (1933-35) that the injection of various types of colloidal materials was followed by an extreme thrombocytopenia due to a sweeping up of the colloidal particles by all the platelets in the circulation, and the resultant massing of agglutinated platelets as thrombi. This was followed by active regeneration of platelets from megakaryocytes, as described by Volterra.³⁹

Other origins of the platelets have been described. Brown⁴¹ in 1913 found that under conditions of excessive demand, platelet production from monocytes could occur. Bunting³⁶ in 1920 claimed that in epidemic influenza in which thrombocytopenia was present, platelets or platelet-like bodies were formed from lymphocytes in the blood stream. Howell and Donahue⁴² (1937), on the basis of arterial and

venous platelet counts, histologic studies, and perfusion experiments, concluded that new platelets were added to the blood in capillary areas of the lungs and were destroyed in the systemic capillaries

Originally based on purely morphologic grounds, the evidence from both histologic and experimental studies that platelets are formed from the megakaryocytes of the bone marrow can hardly be more complete. Under abnormal conditions, as in myeloid metaplasia, these giant cells may also develop in the spleen, the lungs, and in other sites. Vicarious platelet production from lymphocytes, monocytes, etc., is a possibility under conditions of unusual demand. The literature on this subject is carefully reviewed by Tocantins.⁴³

B Variations in the Blood Platelet Count, Certain Regulatory Mechanisms—The number of platelets in the circulating blood probably depends upon several factors, none of which has been adequately studied. Probably of greatest importance is the total number of megakaryocytes and their degree of activity. In conditions with greatly increased megakaryocytes, as in polycythemia vera and certain cases of chronic myelogenous leukemia, the platelet count becomes considerably increased. Megakaryocytic activity is also stimulated by excessive blood loss, whether due to hemorrhage or to increased hemolysis. The operative removal of various organs, such as the uterus, the stomach, etc., is followed by a transient thrombocytosis,⁴⁴⁻⁴⁶ which may, however, be a function of bleeding rather than dependent upon actual removal of the organ. When megakaryocytes are conspicuously reduced, as in acute leukemia, aplastic anemia, destructive lesions of the bone marrow, and certain deficiency disorders such as pernicious anemia, the blood platelets are also greatly diminished. In these abnormal situations, the relationship of megakaryocytes to platelets seems to be clear and well defined. However, no such clarity exists when one considers the normal blood platelet count. The mechanisms dealing with the stimulation of platelet growth and budding, the delivery of platelets from the bone marrow to the blood, the life span of the platelet, and its mode of destruction and disintegration remain quite obscure.

As noted above, a potent stimulator of megakaryocytic and platelet production is blood loss, whether by hemorrhage or by hemolysis. Since blood destruction by hemolysis is constantly taking place, it is possible that this process results in a constant stimulation of megakaryocytic platelet growth. In women, the menstrual cycle appears to be closely related to variations in the count. With onset of the catamenia, as one of us⁴⁷ showed a number of years ago, there is an immediate and striking rise in platelets to about twice their original level. This may be interpreted as indicative of an endocrinal relationship, but it may simply be due to the stimulating effect of hemorrhage into the endometrial mucosa and its sudden separation from the uterus. In any event, the platelet count of menstruating women is subject to regular peaks and valleys, determined largely by the menstrual cycle, and is quite in contrast with that of men and nonmenstruating women. The low megakaryocyte and platelet counts in pernicious anemia, and the prompt response in platelet count following treatment with liver extract—often in advance of the reticulocyte increase—suggest that liver extract substance is required for megakaryocyte platelet growth.

The mechanisms which have to do with the *delivery* of platelets from the marrow to the blood are also obscure. One fact is, however, certain: the operative removal of the normal spleen is followed, not only by a marked temporary increase in platelet count, but by a sustained high platelet level which may be present as long as twenty years after splenectomy.⁴⁷ This is quite in contrast with the transient platelet increase which occurs following the removal of an organ such as the uterus, and suggests that the normal spleen exerts a regulatory (inhibitory?) effect on either the growth of the platelets or their delivery from the marrow to the blood. Support for this hypothesis is given by the findings in many cases of splenomegaly, whether due to cirrhosis of the liver, splenic vein thrombosis, chronic infection, Gaucher's disease, Boeck's sarcoid, Felty's syndrome, or whatever.⁴⁸ In these conditions, leukopenia, granulocytopenia, and thrombocytopenia are usually present, suggesting that the large spleen exerts an unusually marked inhibitory effect on both leukocytic and platelet formation and/or delivery.⁴⁸ Evidence indicating that the thrombocytopenia is due to an inhibition of delivery rather than to disturbed formation is brought out by the findings in the bone marrow which show an increase in megakaryocytes with a normal degree of platelet production (table 5). Following splenectomy in these cases, the platelets rise quickly to high levels, where they remain indefinitely.

C. *The Marrow Findings in Idiopathic Thrombocytopenic Purpura*—The above observations lead us to interpretations regarding the marrow findings in essential or idiopathic thrombocytopenic purpura and their relationship to the spleen and splenectomy. Frank⁶ in 1915 was the first to hypothesize that the low platelet counts might be due to a dysfunction of the megakaryocytes. His actual studies of the bone marrow were reported fully in 1925¹⁶ in his authoritative review of the hemorrhagic diseases in Schittenhelm's *Handbuch der Krankheiten des Blutes und der Blutbildenden Organe*. In this article, he noted diminished granularity and greatly diminished platelet production from megakaryocytes, together with the presence of degenerative changes. The article is illustrated with several excellent drawings in color. Frank's very complete studies have unfortunately received little attention, particularly in this country, where the concept of an undue thrombocytolysis by the spleen seems to have gained wide credence. This concept, which was originated by Kaznelson⁷ of Prague in 1916, was based on the finding of an enlarged spleen in a case of chronic thrombocytopenic purpura; studies of the bone marrow were not made. In 1917, Minor,⁴⁷ with the help of J. H. Wright, studied the bone marrow of a fatal case of the disease. The megakaryocytes were plentiful and perhaps even slightly increased above normal. From the available preparations, we could not tell if there was any definitely altered histologic appearance of the cells. These important statements were made. We can suppose that, though these giant cells are plentiful in numbers, they became affected so that they are unable to allow platelets to be cut off from them in normal fashion. Is it not possible that at times, with or without hypertrophy of the spleen, its physiologic activity is altered so as to cause bone marrow depression? Seeliger⁴⁸ (1924) in 2 cases and Gasper⁴⁹ (1926) in 1 case found increased megakaryocytes. Weiner and Kaznelson⁵⁰ in 1926 stated that megakaryocytes were abundant in all

their cases. Since they found the structure of the megakaryocytes normal, they postulated an unusual thrombolytic function of the spleen. They found more platelets in smears made directly from the extirpated spleen than in the splenic blood. No actual evidence of thrombocytolysis was, however, brought forth. Jedlicka and Altschuler⁵¹ in 1925 studied the marrow from 2 cases of chronic essential purpura. The megakaryocytes were numerous and showed increased vacuolization and deficiency in granularity. In an autopsied case, Schmincke⁵² found increased megakaryocytes, their cytoplasm was free of granules and many contained leukocytes and lymphocytes. The latter finding was interpreted as

TABLE 7—*Acute Idiopathic Thrombocytopenic Purpura—Differential Megakaryocyte Counts Before and After Splenectomy*

Case	Dates	Blasts	Promega karyo cytes			Lym phoid		Inter me diate		Adult		Degenerated forms				Mitotic figures	Megakaryocytes per million nuc cells	Per cent age with pits	Blood pits per cu mm	State
			s pits	e gran pits	nongran pits	e pits	s pits	e pits	s pits	e pits	s pits	Eosin	Vacuol	Hyal	Loss gran					
A K	9-14-40	2	9	1	7	1	7	0	5	0	35	2	1	0	14	1	546.9	18	45 000	Before Rx
	9-23-40	6	7	1	16	0	3	5	21	0	12	0	0	0	13	0	449.4	22	59 000	
	10-2-40	0	0	6	2	6	10	0	0	30	34	0	0	0	12	0	327.6	42	292 300	X-ray Thee- lol
	10-13-40	0	4	6	0	6	8	10	0	46	12	0	4	0	4	0	739.8	68	459 000	48 hrs post spl
L Z	5-10-40	1	5	9	6	1	9	0	0	9	45	3	3	0	9	0	501.3	19	25 000	Before splenec
	5-16-40	0	2	15	2	1	3	0	0	50	13	0	0	0	8	2	324.3	72	111 000	24 hrs post
	5-17-40	0	6	12	0	0	0	0	0	58	12	0	4	0	10	0	—	60	242 000	48 hrs post
I G	8-3-38	2	9	1	3	3	1	0	9	5	50	0	2	0	9	2	743.9	9	20 000	Before splenec
	8-9-38	0	0	41	0	3	0	9	0	32	6	0	0	0	9	0	898.6	85	440 000	5 days post
R L	8-18-39	8	8	9	13	3	4	5	4	1	14	1	3	0	17	1	366.4	18	15 000	Before splenec
	8-24-39	6	4	30	0	6	2	3	0	30	8	0	1	0	10	0	408.1	69	240 000	24 hrs post
	10-3-39	0	11	2	1	3	9	4	3	13	41	0	2	0	11	0	444.9	22	154 000	Mild re- lapse

evidence of phagocytosis. Gerlach⁵³ reported an autopsied case with numerous megakaryocytes in the marrow. Large pyknotic nuclei, with little cytoplasm, containing few or no granules were present, pseudopods were rare. Nickerson and Sunderland,⁵⁴ studying autopsy material, found that the megakaryocytes were either increased or decreased in number. The predominance of young forms suggested a functional hyperplasia. These observers found that the platelets and the megakaryocytic granules were very fragile and unusually susceptible to destruction both by postmortem change and the various processes involved in fixing the hydrating tissues prior to staining.

Studying the sternal biopsies of 4 cases of idiopathic thrombocytopenic purpura, Willis⁵⁵ believed that the megakaryocytes were not pathologic, however, no

platelet formation was seen. In 1 case undergoing spontaneous recovery, the peripheral platelet count having reached 207,000 per cu mm, Krjukof⁵⁶ found 17 marrow biopsy numerous normal appearing megakaryocytes surrounded by large numbers of platelets.

Klima⁵⁷ studied 17 cases by the method of sternal puncture. Megakaryocytes were found increased in the majority. This was particularly true of the chronic cases, in which nongranulated megakaryocytes were often found. Rosenthal⁵⁸ stated that the megakaryocytes are present in normal numbers or increased in thrombocytopenic purpura hemorrhagica. The diminution in platelets is either due to increased destruction in the spleen, or is the result of lack of fragmentation of the megakaryocytes. Scott⁵⁹ investigated the sternal marrow in 1 case and stated that although the megakaryocytes were certainly more abundant in most films, qualitative changes in the cells were not striking.

Recently Limarzi and Schleicher,⁶⁰ employing 1 technic whereby films are made from the nucleated cell layer from the sternal marrow at puncture, found a marked megakaryocytic hyperplasia. In the acute cases younger forms of megakaryocytes predominated, while in the less acute phases adult types appeared to be increased. Pathologic or toxic forms of megakaryocytes, cells with degenerative types of nuclei and hyaline cytoplasm and an absence of azurophilic granules, were frequently observed. Splenectomy resulted in a reversion of the marrow picture to normal values. They concluded that splenectomy removed 1 factor inhibitory to the maturation of megakaryocytes and that the differential diagnosis of purpuric states could be satisfactorily made from bone marrow studies.

Fieschi and Villalobos,⁶¹ studying the bone marrow in 8 cases, found changes in the megakaryocytes consisting of a disturbance in maturation of the cells and of a deficiency in platelet formation and detachment. There was also 1 lack of platelets in the blood of the marrow. The megakaryocyte counts showed an increase in young forms and an asynchronism in the maturation of the nucleus and protoplasm. Distinct hyperlobulation was present. Degenerated forms were inconstant and never very numerous.

Lawrence and Knutti,⁶² in 6 cases of idiopathic thrombocytopenic purpura, found the bone marrow sections normal in number in 4 cases. In the other 2, the megakaryocytes were decreased. Morphologic variations existed in the megakaryocytes of 4 of the cases. These authors concluded that at least two different varieties of the disease were present and suggested that splenectomy was of greater value in those cases with normal bone marrows. On the other hand, Heindl⁶³ in 4 cases was unable to demonstrate qualitative characteristic alterations of the megakaryocytes. Wiseman, Doan, and Wilson⁶⁴ reported bone marrow findings before and after splenectomy and found practically no change. The numbers of megakaryocytes are given but are not compared with normals, and the time intervals after splenectomy are not stated.

The majority of the reports in the literature thus give the impression that the megakaryocytes are increased in essential thrombocytopenic purpura. The only real attempt to compare their numbers with those of normal cases is that of Limarzi and Schleicher,⁶⁰ who found a definite increase. With reference to qualitative

changes, most reports deal with fixed material, usually obtained at autopsy. Here, unless special care is taken in technic, the platelets and granules are at least partially destroyed, and classification of the types of megakaryocytes becomes very difficult. For the proper study of the megakaryocyte pattern and platelet production, the use of biopsy material with the preparation of well spread, well stained smears directly from touch or aspirated material is essential.

Our own observations of the marrow in idiopathic thrombocytopenic purpura agree closely with those of Frank,¹⁶ who found a great diminution in megakaryocytic platelet production corresponding with the marked platelet diminution in the blood. However, since the actual number of megakaryocytes is often greatly increased, although very few of them are producing platelets, the total concentration of platelet-producing tissue may not be as severely depleted as would appear at first glance. This might indicate not only a disturbance in the megakaryocyte platelet production, but an inhibition of delivery as well.

D *The Nature of Idiopathic Thrombocytopenic Purpura*—Several basic facts are paramount in any discussion of the nature of the disease known as idiopathic or essential thrombocytopenic purpura.

- 1 The blood platelets are considerably reduced
- 2 The bone marrow shows normal or increased numbers of megakaryocytes
- 3 The megakaryocytes show a greatly diminished productivity of platelets
- 4 Splenectomy leads in most cases to a great increase in platelet production by megakaryocytes, and
- 5 To a great increase in blood platelets

At first glance, it might appear paradoxical that the blood is practically depleted of platelets while the bone marrow contains normal or even increased numbers of megakaryocytes. Careful inspection of the megakaryocytes reveals what appears to be an adequate explanation of this discrepancy: the megakaryocytes show very little if any platelet production, which is often abnormal in type, proceeding from immature forms. The low blood platelet count is therefore in all probability due to a diminished production of platelets from megakaryocytes, although a diminished delivery of platelets from megakaryocytes may also be a factor. The qualitative changes in platelets as seen in the blood—large forms, often bizarre in shape—may be due to their abnormal production by the earlier forms of megakaryocytes.

These observations must be aligned with the undeniably dramatic effects of splenectomy. Often within an hour, and almost always within a few hours after this operation, the platelet count begins to rise and quickly reaches abnormally high values. Simultaneously the marrow shows striking changes: within a few hours platelet budding is seen proceeding from most megakaryocytes, within two days huge masses of platelets are often seen overrunning whole microscopic fields.

If these observations are correct, it would appear that only one conclusion as to the pathogenesis of the disease is tenable: i.e., that *it is due fundamentally to an abnormality of the spleen, which exerts an unusual effect upon the production of platelets from the megakaryocytes in the marrow*. This postulates a hormonal relationship between the spleen and the bone marrow. Several indications of such a relationship

are already at hand ⁴⁷⁻⁴⁸ (1) following splenectomy in the normal human and animal the white cell and platelet counts rise and Howell-Jolly bodies appear in the red cells, (2) in many conditions associated with a large spleen, the leukocyte, granulocyte, and platelet counts usually are diminished, although the bone marrow is hyperplastic, (3) following splenectomy in such conditions, the leukocyte, granulocyte, and platelet counts rise to normal or high values. These findings suggest that the spleen may secrete materials with effects upon the production of red cells, granulocytes, and platelets in the marrow and their delivery to the circulating blood. Direct proof of such a hormonal relationship has thus far not been established, although a number of observers in the past few years have demonstrated a possible platelet-inhibiting hormone in acetone extracts from the spleens of cases of idiopathic thrombocytopenic purpura ⁹⁻¹⁵. Recent experiments in our laboratory ⁶⁵ have demonstrated that the injection in dogs of homogenized saline extracts of fresh whole spleen from cases of idiopathic thrombocytopenic purpura is followed by an extreme reduction in the platelet count, the marrow simultaneously showing very large numbers of megakaryocytes.

The hypothesis originally advanced by Kaznelson⁷ that the spleen required removal because of its unusual thrombolytic effect is unconvincing, since histologically the spleen shows little, if any, deviation from the normal. Frank¹⁶ attacked the thrombolytic hypothesis vigorously. How, he asked, can the sudden platelet increase take place unless a sufficient number of mother cells are already present in the marrow ready to take on platelet formation? Furthermore, Frank went on, actual thrombocytolysis in the extirpated spleen was never demonstrated, even by Kaznelson. The splenic morphology is often diverse in the presence of the thrombocytopenic condition. The more recent studies of Wiseman, Doan, and Wilson,⁶⁴ utilizing the supravital technic and seeming to point to a phagocytic activity of the splenic clasmatoocytes upon platelets, require confirmation.

Allergic states, in which there is a direct effect upon the megakaryocytes without splenic mediation, may be at the basis of some cases of thrombocytopenic purpura ⁶⁶⁻⁶⁷. By and large, however, idiopathic thrombocytopenic purpura must be considered as primarily a disease of the spleen with secondary effects upon the bone marrow megakaryocytes, thus resulting in a disorder of the blood. The disease may thus be considered a form of hypersplenism or hypersplenic thrombocytopenia, and as such may be compared with the hypersplenism of splenic neutropenia ⁶⁸⁻⁷⁰. The cause of the abnormal splenic activity and what initiates it still remain obscure. Were this obscurity to be lifted, it might be possible to modify the action of the spleen without actually having recourse to its removal.

E. The Diagnostic Value of the Bone Marrow Biopsy in Thrombocytopenic Purpura — A reduction in platelets—thrombocytopenia—may be due to various mechanisms which may be classified as follows. (1) Actual disease of the entire bone marrow—aplasia, hypoplasia, infiltration by abnormal cells or tissues, fibrosis, liver extract deficiency, etc., here the platelets are reduced, together with the granulocytes and the red cells, and pancytopenia is usually present. (2) Selective involvement of the megakaryocyte platelet mechanism by the action of chemicals, infections, and allergens. (3) The hypersplenism of many cases of splenomegaly. Gaucher's

disease, Felty's syndrome, Boeck's sarcoid, cirrhosis of the liver, etc., in these cases leukopenia and granulocytopenia are also common (4). The abnormal hypersplenism of idiopathic thrombocytopenic purpura. The differential diagnosis between these conditions usually offers little difficulty, but occasionally one is hard put to it to discriminate definitely between an idiopathic case and one which is secondary or symptomatic of a more fundamental disorder. Since this may be important from the standpoint of splenectomy, the diagnostic value of the bone marrow biopsy must be considered.

In the first group, which may be called secondary thrombocytopenia, the bone marrow shows either aplasia, hypoplasia, leukemic infiltration, sarcomatous invasion, or fibrotic replacement. The megakaryocytes are conspicuously diminished, in extreme cases, none of these cells is seen. The great reduction in these cells is the outstanding feature which differentiates the condition from the idiopathic disease, the few remaining megakaryocytes are essentially normal in size, shape, appearance, and platelet production.

In the allergic type, and in infectious thrombocytopenias, the megakaryocytes are selectively involved, while the rest of the marrow is essentially normal. Schwartz⁶⁷ has shown that bone marrow eosinophilia is a common finding in the allergic group, even in the absence of blood eosinophilia. In the toxic and infectious types, there may be a definite reduction in the megakaryocytes, with the granulocytes often showing marked toxic changes.

In the symptomatic thrombocytopenia of hypersplenism associated with a large spleen, the megakaryocytes are numerous and platelet production appears to be normal. The low platelet count and the response in platelets following splenectomy must therefore be due either to an inhibition of delivery of platelets from the marrow to the blood by the unusually active enlarged spleen, or to unusual phagocytosis of platelets by that organ. Our histologic studies of the spleen indicate that the first of these explanations is probably the correct one.

In the idiopathic group there is usually an increase, often great, in the megakaryocytes, which, however, show greatly diminished platelet production. The ability to recognize this abnormality may be of prime importance in deciding whether splenectomy will be of value in a given case.

SUMMARY AND CONCLUSIONS

1. The megakaryocytes of the sternal bone marrow at biopsy were studied in 11 cases of idiopathic thrombocytopenic purpura and compared with those of 10 normal cases, 5 of thrombocytopenic purpura associated with various types of splenomegaly, and of a large group of miscellaneous hematologic conditions, including leukemia, associated with a reduction in platelets.

2. Megakaryocyte counts expressed in terms of a million nucleated red cells and differential counts of megakaryocytes were performed. The megakaryocytes were classified as megakaryoblasts, promegakaryocytes, and mature forms, and were further subdivided into those showing granularity, platelet production, degenerated forms, and mitoses.

3. In the normal cases, not more than 300 megakaryocytes per million nucleated red cells were present, and an average of 68.6 per cent showed platelet production.

4 In acute idiopathic thrombocytopenic purpura, although the platelets in the circulating blood were rare, megakaryocytes were increased, being present in a proportion of 366 to 743 per million nucleated red cells. Platelet production was, however, greatly diminished and found in only 8 to 19 per cent of all megakaryocytes. Following splenectomy, there was a striking increase in platelet production, which was now present in 69 to 85 per cent of all cells, the large masses of new platelets in the marrow were often very striking.

5 In chronic idiopathic thrombocytopenic purpura, the megakaryocytes were considerably increased over normal values, but showed great diminution in platelet production, following splenectomy, extreme degrees of platelet production from megakaryocytes took place.

6 In splenomegaly of nonleukemic origin (cirrhosis, splenic vein thrombosis, Gaucher's disease, Felty's syndrome), the megakaryocytes were somewhat increased, but platelet production was normal.

7 In aplastic anemia, lymphosarcoma, acute leukemia, and other diseases invading or destroying the bone marrow, the megakaryocytes were conspicuously reduced, the few remaining cells present being of normal morphology.

8 The origin of the blood platelets from megakaryocytes, certain regulatory mechanisms for platelet production and delivery, and the possible relationship of the spleen to these mechanisms are discussed.

9 The findings of increased megakaryocytes and greatly diminished platelet production in the marrow before splenectomy and the striking increase in platelet production after splenectomy indicate a definite pathogenetic relationship of the spleen to the disease. Idiopathic thrombocytopenic purpura is probably a form of hypersplenism (splenic thrombopenia) in which, through a possible hormonal mechanism, the megakaryocytes of the bone marrow are inhibited from normal platelet production and delivery.

10 The marrow findings in idiopathic thrombocytopenic purpura are sufficiently characteristic to be of diagnostic value in differentiating the disease from leukemia and other conditions associated with a low blood platelet count.

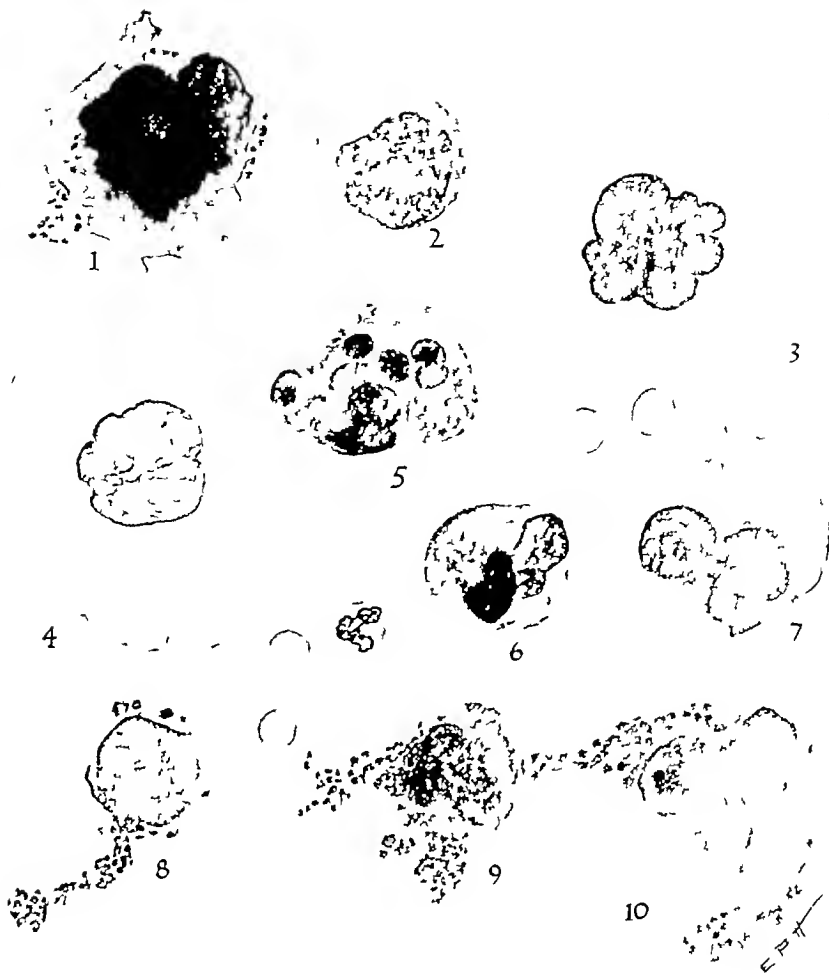
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1 Mature megakaryocyte Note granularity of cytoplasm, pseudopods and the presence of platelets, which are situated chiefly at the periphery of the cell and in the pseudopods

2 Megakaryoblast

3 Mature megakaryocyte or intermediate form, from a case of chronic idiopathic thrombocytopenic purpura There is well defined granularity of the cytoplasm, but no platelet formation

4 Mature megakaryocyte from a case of chronic idiopathic thrombocytopenic purpura There is almost complete lack of granularity and marked vacuolization and degeneration of the cytoplasm

5 Intermediate form of megakaryocyte from an acute case of idiopathic thrombocytopenic purpura Granule formation, without platelet development is evident and there are some questionable nuclear bodies (asynchronism of development) in the cytoplasm

6 7 Lymphoid megakaryocytes from an acute case of idiopathic thrombocytopenic purpura These are characterized by blue cytoplasm, lack of granularity and lack of platelet formation

8 Promegakaryocyte from an acute case of idiopathic thrombocytopenic purpura 24 hours after splenectomy Nongranular platelet formation is present around the periphery of the cell with a streamer containing a group of newly formed platelets

9 10 Intermediate form and mature megakaryocyte from an acute case of idiopathic thrombocytopenic purpura 48 hours after splenectomy There is a striking productivity of granular (functioning) platelets seemingly with the entire cytoplasm almost ready to break up into platelets

All illustrations drawn with the aid of a camera lucida at a magnification of 1200 times

THE VALUE OF PENICILLIN IN THE TREATMENT OF AGRANULOCYTOSIS CAUSED BY THIOURACIL

By MARY CATHERINE TYSON, M D , PETER VOGEL, M D , AND NATHAN
ROSENTHAL, M D

MACKENZIE and McCollum¹ found that the use of sulfonamides in experimental animals produced thyroid hyperplasia and insufficiency Astwood² tested more than 100 similarly related compounds which might inhibit the function of the thyroid gland, and found that thiourea and thiouracil produced this effect From the clinical point of view these substances seemed to be the most promising and the least toxic of the effective drugs *

According to Astwood,³ thiouracil exercises its effect through the inhibition of the formation of thyroid hormone by some type of enzymatic activity As a result of the depletion of thyroxin, the anterior lobe of the pituitary gland is stimulated to produce more thyrotropic hormone, causing thyroid hyperplasia which is ineffective in the presence of continued thiouracil administration According to previous studies made by others, and at our hospital by Gabrielove, Kert, and Soffer,⁴ it is apparent that the drug is very effective in reducing the basal metabolic rate and bringing about clinical improvement in cases of Graves disease The usual therapeutic dose is one gram daily in divided doses After four weeks, this is reduced to one tenth or two tenths of a gram daily for maintenance purposes A smaller dose than this (0.1 gram) has recently been recommended

During the course of his preliminary experiments, Astwood⁵ noted that one of his patients developed leukopenia This followed the administration of the drug for approximately thirty-six days, at the end of this period there was malaise and a temperature of 105° The total white cell count fell to 1,100 per cubic mm with complete absence of granulocytes The drug was then discontinued and the patient was given sulfathiazole, liver extract, and pentnucleotide, and recovery took place one week later The toxic effect of the drug on the blood was thought to be due to the large dose of thiouracil employed In this instance, two grams daily were administered for eight days

Our attention was called to a somewhat similar case by Dr M A Rubinstein⁶ In this instance the toxic effect of the thiouracil was more prolonged The patient developed marked granulocytopenia but recovered completely after several weeks with the aid of transfusion therapy There have been a number of reports in which some of the patients developed moderate leukopenia, generalized lymphadenopathy, skin sensitivity, and fever after eight to ten days of administration of the drug These symptoms were followed by complete recovery when the drug was discontinued Additional cases of agranulocytosis have been reported together with one fatal case by Kahn and Stock⁷

The purpose of this paper is to report nine additional cases of severe agranulo-

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* Thiouracil is produced by Lederle Laboratories under the trade name Deracil (2-thio 6-oxypyrimidine)

cytosis following the use of thiouracil and to stress the effectiveness of penicillin in the treatment of this dangerous complication

Of the nine cases studied, four were fatal, five recovered after penicillin therapy. The first patient died forty-eight hours after admission to the hospital, but did not receive penicillin. The second patient was treated with penicillin but succumbed to an underlying cardiac condition. The third patient succumbed in spite of what was thought to be an adequate dosage of penicillin. The fourth patient died ten hours after admission to the hospital, after only one day of treatment. The remaining five patients all recovered following the use of penicillin.

The case histories will be given in abstract form and only the pertinent data mentioned, since some will be reported in greater detail subsequently by Gabrilove, Kert, and Soffer.⁸

FATAL CASES

Case 1. D. B. was a 49 year old Austrian born widow who entered the hospital for the first time on January 15, 1944, with a two year history of increasing nervousness, tremor of the hands, protrusion of the eyes, dyspnea, palpitation, profuse perspiration, and weight loss of 63 pounds. On physical examination the patient showed marked exophthalmos, lid lag and stare, and a fine tremor of the hands. The thyroid gland was uniformly enlarged. The patient was apprehensive, emotional and hyperkinetic. Her heart was overactive with a rate of 125 beats per minute. Two basal metabolic rates on successive days were plus 75 per cent and plus 77 per cent.

The blood picture was as follows: hemoglobin 77 per cent, white blood cell count 6,400 per cubic mm with the following differential: segmented polymorphonuclear leukocytes 52 per cent, nonsegmented polymorphonuclear leukocytes 2 per cent, lymphocytes 34 per cent, monocytes 6 per cent, eosinophiles 6 per cent. The patient was given 0.2 grams of thiouracil orally five times a day beginning on January 21.

Twelve days later, February 1, the basal metabolic rate had fallen to plus 30 per cent and the pulse rate to 80 per minute. The patient felt greatly improved, seemed much quieter and had gained 5 pounds in weight. On February 21, after thirty-one days of thiouracil therapy, she had gained 12 pounds and showed little evidence of hyperthyroidism. The basal metabolic rate was now plus 5 per cent. The hemoglobin concentration was 77 per cent, the white cell count 5,800 per cubic mm. During this period the white blood cell count varied between 5,800 and 6,800 with normal differential counts. The patient was discharged to a convalescent home, where she continued to receive 0.4 grams of thiouracil daily until March 2, receiving a total of 33.6 grams within forty days. On the fortieth day of thiouracil she developed a cough, fever and a shaking chill. Thiouracil was discontinued and aspirin was given. A blood smear taken at the convalescent home on this day, was not examined, however, until three days later and found to have the following differential: segmented neutrophils 6 per cent, nonsegmented neutrophils 2 per cent, lymphocytes 90 per cent, and monocytes 2 per cent.

The patient was readmitted to the hospital on March 5, acutely ill and febrile with a diffusely red throat and a macular skin rash. The white blood cell count was 360 per cubic mm and only lymphocytes were found on the smear. Penicillin was not available at this time. A transfusion of 500 cc of citrated blood was given followed by sulfamerazine by continuous intravenous infusion. On March 6, the patient's temperature ranged between 105° and 106°. The blood culture was negative. She became deeply icteric and the liver became tender and enlarged to three fingers breadth below the costal margin. The hemoglobin was 90 per cent with 4.42 million red cells per cubic mm, the platelets were 150,000 per cubic mm, the white cells numbered 800 per cubic mm and all were lymphocytes.

Sternal marrow aspiration revealed only 11,000 nucleated cells per cubic mm and showed the following differential count:

Myeloblasts	8 per cent	Lymphocytes	20 per cent
Hematogones	10 per cent	Plasma cells	10 per cent
Reticulum cells	20 per cent	Normoblasts	30 per cent
Megakaryocytes	2 per cent		

This revealed a hypoplastic marrow with a disappearance of all mature and most of the immature granulocytes. As in other very severe cases of agranulocytosis, there was a relative increase of reticulum cells, lymphocytes and plasma cells.

The patient died in a state of hyperpyrexia on March 7, two days after the second admission to the hospital. The essential findings revealed at the postmortem examination were jaundice, a hyperplastic thyroid, esophagitis with erosion of the lower third of the esophagus, and cloudy swelling of the liver. Postmortem marrow smears were similar to those obtained by sternal aspiration the day before death.

*Case 2.** N S, a 59 year old woman, was treated for arteriosclerotic heart disease, arricular fibrillation, thyroid adenoma and diabetes mellitus for one year. Her first basal metabolic rate was plus 43 per cent. In December 1943 it had risen to plus 80 per cent but operation was deemed inadvisable because she was considered to be a poor surgical risk. She was given 0.2 grams of thiouracil six times a day for three weeks when it was reduced to 0.1 grams six times a day and continued for three weeks longer. A total of 37.8 grams was given over a period of six weeks. Repeated blood counts were normal. On February 1, 1944 acute tonsillitis developed and streptococcus hemolyticus was found on culture. Six grams of sulfadiazine were given daily for eighteen days. At the end of this period of medication on February 19 the white blood count was 8,500 per cubic mm, 65 per cent of which were polymorphonuclear cells. The signs and symptoms of hyperthyroidism increased markedly and on February 25 thiouracil was started again with a dosage of 1.2 grams daily for ten days. A total of 12.5 grams was given. She was discharged improved from the hospital on March 6 with a white blood count of 8,650 cells per cubic mm and 70 per cent polymorphonuclear cells. One week later she was readmitted with fever, cellulitis of the face and ulcerative lesions of the tonsils and pharynx. On examination of the blood an extreme leukopenia (250 cells per cubic mm) was found. No granulocytes were seen and only lymphocytes were found on the blood smears. On admission 2 grams of sulfadiazine were given. Penicillin was then obtained, and 120,000 units were given daily. Blood counts performed by one of us called in consultation on March 13 revealed the following:

Hemoglobin	69 per cent
Red blood cells	3.86 million per cubic mm
White blood cells	200 per cubic mm, all lymphocytes
Platelets	110,000 per cubic mm

A sternal marrow aspiration done simultaneously showed the following:

Total nucleated count—32,000 per cubic mm

Megakaryocytes—22 per cubic mm

Myeloblasts	3 per cent	Lymphocytes	4 per cent
Promyelocytes	1 per cent	Plasma cells	7 per cent
Megakaryocytes	1 per cent	Erythroblasts	10 per cent
Reticulum cells	16 per cent	Normoblasts	35 per cent
Hematogones	23 per cent		

The bone marrow showed the typical findings of agranulocytosis. The patient's condition became worse and she died in cardiac failure on March 17 after 680,000 units of penicillin had been given.

Case 3. F K was a 47 year old native born woman with a negative past history. Eight weeks before admission she developed dyspnea, tachycardia, exophthalmos and palpitation. A basal metabolic rate at this time was plus 65 per cent. She was given thiouracil 0.6 grams daily for two weeks. At that time signs and symptoms of Graves' disease had regressed, the patient had gained weight and the basal metabolic rate was plus 31 per cent. She was then given 0.2 grams of thiouracil daily until admission at which time she had had a total dosage of 16.8 grams in eight weeks. Blood counts were done twice a week by her physician, and the last one three days before admission was reported as normal.

The day before admission she developed a sore throat and fever. On the day of admission, November 12, 1944, a blood count revealed only 1,000 white cells per cubic mm, all of which were lymphocytes.

* We are indebted to Dr. Ralph Sussman for permission to publish this case.

Physical examination revealed an acutely ill middle-aged female with a temperature of 105°F and a markedly reddened throat with enlargement of the anterior cervical nodes. Lungs, heart, and abdomen were normal. White blood cell count was 750 per cubic mm and only lymphocytes were found on the blood smear. Hemoglobin was 85 per cent, red cell count 4.75 million per cubic mm, and platelets 300,000 per cubic mm. Sternal marrow aspiration yielded 60,000 nucleated cells per cubic mm, with 44 megakaryocytes per cubic mm.

Differential count

Myeloblasts	3.6 per cent	Reticulum cells	2.4 per cent
Myelocytes	4.0 per cent	Erythroblasts	1.6 per cent
Lymphocytes	4.0 per cent	Normoblasts	53.6 per cent
Hematogones	5.2 per cent	Megakaryocytes	0.4 per cent
Plasma cells	5.0 per cent		

This showed a marked decrease in myeloid cells with no mature granulocytes together with an increase in nucleated red cells, lymphocytes, plasma cells, and reticulum cells, some of which were vacuolated.

Penicillin therapy was started immediately after admission in dosage of 20,000 units every three hours intramuscularly. In addition, a transfusion of 500 cc of whole blood, continuous intravenous glucose solution in saline with added vitamins were given. On November 13, 1944, icterus was present and the urine showed three plus bile and no urobilin. The white blood count was 500 cells per cubic mm—all lymphocytes. The pharynx showed marked reddening and the temperature of 105°F persisted. On November 14, 1944, the icterus deepened, the liver was smaller on percussion, and the patient appeared moribund. Blood culture revealed the presence of *Streptococcus viridans*, reported as being ten times as resistant to penicillin as the standard organism. Icterus index was 60. The white blood cell count was 500 per cubic mm—all lymphocytes. On November 15, her condition was unchanged. A blood count revealed 600 white cells per cubic mm, with 88 per cent lymphocytes, 8 per cent monocytes, and 4 per cent segmented polymorphonuclear cells. The hyperpyrexia continued and the patient showed signs of meningeal irritation. She died a few hours later. Necropsy showed a necrotizing pharyngitis and a toxic hepatitis.

Case 4. E. C. a 32-year-old female was admitted to the hospital on July 3, 1945, with a three-year history of tremors, nervousness, and fatigue. There had been considerable loss of weight in the past year. Five months before admission these symptoms became markedly aggravated and the basal metabolic rate was recorded as plus 80 per cent. Starting on April 18, the patient was given thiouracil, one tablet three times daily until June 18. The drug was discontinued at this time when she telephoned her physician that she felt nauseated. Blood counts had not been done periodically. Four days before admission she developed a sore throat, fever, and chills. Her neck became swollen.

She was admitted to a hospital in a neighboring state and was given 200,000 units of penicillin. Because of the seriousness of her illness she was then referred to Mt. Sinai Hospital for admission. Physical examination revealed a desperately ill female with a temperature of 104°F. Her throat was swollen and red, and there was generalized cervical lymphadenopathy. The rest of the physical examination was essentially negative. Penicillin was continued and a transfusion was given, but she continued to go downhill rapidly and died ten hours after admission. Permission for postmortem examination was not obtained. The blood findings revealed the following:

Hemoglobin	91 per cent	Polys eos	4 per cent
Red cells	5,020,000		
White cells	800	Lymphocytes	82 per cent
Platelets	190,000	Monocytes	12 per cent
Polys nonsegmented	0 per cent	Reticulocytes	2 per cent
Polys segmented	0 per cent		

CASES WITH RECOVERY FOLLOWING PENICILLIN

Case 5 M K, a 29 year old female, was admitted to the private pavilion of the Mount Sinai Hospital on June 2, 1944, because of sore gums and fever. Her past history was negative except for kyphoscoliosis at the age of 15 which was treated by means of a cast. At that time a basal metabolic rate was done and found to be low. She took thyroid medication from time to time. About ten months before admission she felt irritable, nervous, and became emotionally unstable. A fine tremor of the hands developed. She was found to have an increased basal metabolic rate and an enlarged thyroid gland. She was given Lugol's solution for a period of time.

Five weeks before admission to the hospital she was given thiouracil 0.6 grams daily, a total of 21 grams in thirty five days. The basal metabolic rate became normal and she improved subjectively. Blood counts were not done. Five days before admission the patient's gums became painful and she became febrile. A few days later she consulted a dentist who referred her to another physician. A blood count showed granulocytopenia, and she was then referred to one of us and was admitted to the hospital. Physical examination revealed swollen eroded gums with a grayish appearance. One small follicle was present on the posterior pharyngeal wall. The submaxillary and right posterior lymph nodes were enlarged. The thyroid gland was diffusely enlarged. She was given 150,000 units of penicillin intravenously daily for four days, then 25,000 units intramuscularly every four hours for three more days. In addition, 1 cc of liver extract was given daily for six days. The temperature ranged between 104° and 106° for five days and then dropped abruptly on the sixth day after admission. The patient made an uneventful recovery. Three transfusions were given on June 2, 3, and 4. The blood findings were as follows:

	Hgb	RBC	WBC	Platelets	NS	S	B	L	M	Plasma	Myelo- cyte
June 1	71	3.49	2,530	340,000		1	1	95		3	
June 2	73	3.8	2,200	280,000				94	1	4	
June 3	79	5.87	600	200,000			1	99			
June 5	80	4.8	1,600	230,000							
June 6	95	5.5	1,800	340,000	1	1		79	13	6	
June 7	93	5.48	2,300	390,000	5	2		63	14	16	
June 8	95	4.88	4,600	320,000	12	8		57	10	12	1
June 10	100	5.32	8,400	410,000	12	42	1	33	9		3
June 12	96	6.16	8,000	720,000	12	40		33	11		4
June 14	103	5.59	8,200	430,000	5	50		37	5		3

NS—Polys Nonseg, S—Polys Seg, B—Polys Bas, L—Lymphs, M—Monos

Sternal marrow aspiration, done on June 8, showed the following:

Total nucleated count 20,000 per cubic mm

Megakaryocytes 22 per cubic mm

Myeloblasts	6 per cent	Hematogones	3 per cent
Promyelocytes	3 per cent	Lymphocytes	16 per cent
Myelocytes neut	38 per cent	Plasma cells	5 per cent
Polys nonseg	19 per cent	Erythroblasts	4 per cent
Polys seg	3 per cent	Normoblasts	2 per cent
Polys bas	1 per cent		

Case 6 A K, a 50 year old single woman, was admitted to the Mount Sinai Hospital on March 28, 1944, for sore throat and fever of twenty four hours duration. Three months previously she went to her physician complaining of weakness, fatigue, loss of weight, anorexia, insomnia, tremor, and sweating of one year's duration. Her basal metabolic rates were plus 43 per cent, plus 63 per cent, and plus 73 per cent in the course of one week. She was given 0.5 grams of thiouracil daily for seven weeks by her physician. Her basal metabolic rate dropped to plus 19 per cent and she showed marked clinical improvement. A blood count done two weeks before admission to the hospital revealed a normal white count and differ-

entail Two days before admission she felt well except for slight fever and she was told by her doctor to omit the thiouracil The following day her temperature rose to 101° , and she developed a sore throat inflammation of the right upper gums and headache The day before admission her white blood count was 3,250 per cubic mm, with 97 per cent lymphocytes and a complete absence of polymorphonuclear cells Physical examination on admission showed a diffusely reddened throat with yellowish exudate on the left tonsil The gums were swollen and grayish in color Other previous blood counts as reported to us were as follows

	Hgb	RBC	WBC	Polys	Lymphs	Monos
	<i>per cent</i>			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
March 6	100	4 76	7,000	65	33	2
March 27	86	4 62	3,250	1	97	2
March 28	80		2,240		98	2

The patient was given daily transfusions of 500 cc of citrated blood together with 12,500 units of penicillin intramuscularly every three hours for four days The temperature ranged from 102° to 103° for three days and then fell to normal The gum and throat lesions cleared and the patient made an uneventful recovery Peripheral blood counts ranged as follows

	Hgb	WBC	Polys Seg	Polys Nonseg	Lymph	Monos
	<i>per cent</i>		<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
March 29	85	1,250	3		96	1
March 30		1,400			98	2
March 31	80	1,700	1		97	2
April 3	105	3,700	29	14	48	9
April 6		7,250	50	6	35	9

Bone marrow studies were as follows

	March 29	March 30	April 3
Total count	15,000	120,000	110,000
Megakaryocytes		110	44
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Myeloblasts	5 0	2 0	2 0
Myelocytes	9 0	18 5	45 2
Myelocytes eos		0 5	0 4
Nonseg polys	2 0		17 2
Seg polys			2 4
Lymphocytes	32 0	13 5	7 2
Hematogones	16 0	12 0	7 8
Monocytes	1 0		
Plasma cells	3 0	4 5	0 8
Reticulum cells	7 0	3 0	
Megaloblasts		0 5	
Erythroblasts	2 0	2 0	0 8
Normoblasts	21 0	43 0	18 0

Case 7 M S was a 43 year old woman, first admitted to the hospital on March 14 1944 with a history of progressive nervousness, perspiration polyphagia heat intolerance, and dyspnea on exertion of three months duration Examination revealed moderate exophthalmos and lid lag, and enlargement of the isthmus and left lobe of the thyroid gland Tachycardia was present, the apical rate being 100, and a coarse systolic murmur was heard over the entire precordium The blood pressure was 145 systolic, 95

diastolic. The skin was warm and moist, and there was a fine tremor of the fingers. She appeared anxious. The basal metabolic rate was plus 48 per cent and stabilized to plus 29 per cent on bed rest and sedation. The blood count showed 81 per cent hemoglobin, red blood cells 4.54 million, white blood cells 6,050 per cubic mm with a normal differential count. The sternal marrow aspiration revealed a total nucleated count of 540,000 per cubic mm with the following differential:

March 18	Total nucleated count		540,000
	Megakaryocytes		352
Myeloblasts	2.4 per cent	Lymphocytes	0.4 per cent
Myelocytes	27.2 per cent	Hematogones	5.2 per cent
Myelocytes eos	0.8 per cent	Reticulum cells	1.2 per cent
Nonseg	22.0 per cent	Erythroblasts	1.2 per cent
Seg	8.8 per cent	Normoblasts	27.2 per cent
Seg eos	0.4 per cent		

Peripheral Blood Counts

	June 10	June 11	June 12	June 13	June 15	June 16
Hemoglobin (per cent)		88	86	101	105	105
Red blood cells		4.7	4.75	5.1	5.5	5.45
White blood cells	1200	950	2400	2800	5600	11300
Platelets		300,000	310,000	310,000	300,000	310,000
Polys nonsegmented (per cent)		10	12	23	14	14
Polys segmented (per cent)		2	14	14	33	41
Polys eos (per cent)		2	2		1	2
Polys bas (per cent)		2		2	1	
Monocytes (per cent)	10	28	6	12	7	5
Lymphocytes (per cent)	90	56	66	43	38	34
Plasma cells (per cent)					2	2
Myelocytes (per cent)				6	4	2
Reticulocytes (per cent)		0.5	0.5		0.5	

Sternal Marrow Studies

	June 10	June 11	June 16
White blood cells	70,000	105,000	475,000
Megakaryocytes	88	132	110
Myeloblasts (per cent)	4.0	3.6	0.2
Promyelocytes (per cent)		61.6	
Myelocytes (per cent)	34.0		32.3
Myelocytes eos (per cent)	2.0		0.33
Nonsegmented polys (per cent)			40.0
Segmented polys (per cent)			6.6
Polys eos (per cent)	2.8	0.4	
Lymphocytes (per cent)	20.4	7.6	3.3
Hematogones (per cent)	7.6	9.2	3.3
Plasma cells (per cent)	1.6	1.2	0.33
Reticulum cells (per cent)	1.2	0.8	
Erythroblasts (per cent)		0.4	0.66
Normoblasts (per cent)	25.6	14.8	8.33
Megakaryocytes (per cent)	0.4	0.4	
Megaloblasts (per cent)			0.33

On March 24 0.2 grams of thiouracil was started five times daily. This dose was continued until April 25. At this time the basal metabolic rate was plus 6 per cent, the heart rate 70 and she showed little evidence of hyperthyroidism except for tremor of the hands. The dosage of thiouracil was then reduced to 0.2 grams once a day, and the patient was discharged much improved on April 30 with a white count of 5,550 per cubic mm and 75 per cent polymorphonuclear leukocytes. She was followed in the Out Patient Department and weekly blood counts were done. The last blood count on May 31, showed 5,100 white cells per cubic mm with 60 per cent polymorphonuclear leukocytes.

Five days before her second admission to the hospital, which was on June 10, she developed fever, cough, sore throat, and swollen gums. Because of this she failed to keep her clinic appointment but stopped taking thiouracil. She had up to then taken 39.2 grams of thiouracil over a period of ten weeks. A local physician gave her 4 grams of sulfathiazole and then 1 gram of sulfamerazine. On her second admission to the hospital she was acutely ill and toxic with a diffusely reddened throat and gums. Her temperature was 102°F. On the night of admission the white blood count was 1,200 per cubic mm with a complete absence of polymorphonuclear leukocytes. Penicillin therapy was begun immediately. 160,000 units were administered daily by intramuscular injection. The following morning she was worse, and auricular fibrillation was present. She had a right basal pneumonia and a yellowish exudate over the tonsils and gums. The temperature hovered around 104°F for five days, while she continued to receive penicillin daily transfusions and liver extract. On the sixth day the temperature dropped to normal and she was clinically well. The peripheral count gradually returned to normal and the total nucleated count of the marrow approached the count of that before thiouracil was started although even at this time there was still a relative myeloid increase.

Case 8 M F A 58 year old white male was admitted to the hospital on April 4 1944 with symptoms of hyperthyroidism and a history of a basal metabolic rate of plus 58 per cent two weeks previously. Physical examination showed a hyperactive male with tremor of the hands enlargement of the right lobe of the thyroid gland, and a systolic murmur over the precordium. The basal metabolic rates were plus 46 per cent and plus 53 per cent. The peripheral blood count was not remarkable. On April 14 thiouracil was started in a dosage of 0.6 grams daily. On May 4 the dose was reduced to 0.2 grams daily. At the time of discharge from the hospital, on May 14 he had gained 10 pounds in weight had no symptoms of Graves disease and showed a basal metabolic rate of minus 3 per cent. The white blood cell count at that time was 7,200 per cubic mm with 57 per cent neutrophils. He continued to take 0.2 grams of thiouracil daily but signs and symptoms of hyperthyroidism recurred and he was readmitted to the hospital on

	Hgb	RBC	Plat	WBC	Seg	Nonseg	Lym	Mono	E
	per cent				per cent	per cent	per cent	per cent	per cent
June 20	82	4.38		6,300	45	2	44	3	3
June 23				4,000	54	5	36	2	3
June 27				3,700	53	2	44	1	
July 3				2,200	21	5	72		2
July 6				1,600	3	4	92		1
July 7				1,600	2	1	97		
July 8	72			1,900	1		99		
July 9	66	3.6	210,000	1,600	1		99		
July 10				1,900	1		99		
July 11	65	3.37	145,000	1,350	1		97	1	1
July 12	68			1,900	1		99		
July 13				2,000	1		99		
July 14	73	4.5	280,000	2,500	4		86	10	
July 15				2,100	2		94	4	
July 17				3,000	20	6	72	2	
July 18				3,500	14	5	78	3	

Bone Marrow Studies

	July 8	July 9	July 13
White blood cells	96,000		80,000
Megakaryocytes	22		22 0
Myeloblasts	2 0	2 0	
Promyelocytes	0 4	1 6	
Myelocytes neut	0 4		36 0
Myelocytes eos	0 8	0 4	0 2
Nonsegmented	0 8	0 8	0 8
Promyelocytes eos	0 4		0 4
Lymphocytes	2 0	4 4	7 2
Hematogones	58 8	36 2	7 6
Plasma cells		1 2	0 8
Reticulum cells			0 4
Megaloblasts			0 4
Erythroblasts	5 6	6 8	1 6
Normoblasts	28 8	56 2	38 4
Megakaryocytes		0 4	

June 19 with a basal metabolic rate of plus 45 per cent. The physical examination was the same as on the first admission. Thiouracil was continued at a dosage of 0.2 grams daily until July 3. At that time he developed leukopenia, low grade fever and a soreness of the neck. The thiouracil was stopped. On July 8 the temperature rose to 105°, the pharynx became red and swollen and a small ulceration of the posterior pillar was seen. He was given 15,000 units of penicillin intramuscularly every three hours. The fever continued until July 14 and then fell to normal, and the redness of the pharynx gradually receded. Penicillin was stopped on July 18. At this time the patient appeared clinically well and the blood count was approaching normal. Peripheral counts were as shown in preceding tabulation.

Case 9. A P, a 53 year old Russian window trimmer was admitted to the Mount Sinai Hospital on July 12, 1944, with a history of palpitation, diarrhea, and nervousness of six months duration. In addition, there was some increase in appetite although there was a weight loss of 5 pounds. The eyeballs were prominent, and occasional diplopia was noted. The skin was warm and moist, there was a systolic murmur at the apex and tremor of the extended fingers. The patient was given 0.2 grams of thiouracil three times a day. Before medication the basal metabolic rate was plus 64 per cent. The blood count on July 13 was as follows:

Hemoglobin	83 per cent	Lymphocytes	33 per cent
Red cells	4,500,000	Monocytes	4 per cent
White cells	8,550		
Polys nonsegmented	6 per cent		
Polys segmented	57 per cent		

The basal metabolic rate gradually dropped to plus 19 per cent. The blood counts were done at periodic intervals, and on the eleventh day of August he was found to have a marked leukopenia and lymphocytosis. The drug was immediately discontinued and he was given penicillin beginning with 10,000 units every three hours, the dose being doubled in two days following a rise in temperature. The fever continued for seven more days. He was desperately ill and finally the white blood count began to increase and the temperature dropped to normal levels. On August 21 he developed a peritonsillar abscess which required incision and drainage. He finally recovered from the agranulocytosis but the hyperthyroidism was essentially unchanged.

Peripheral Blood Counts

	Aug 11	Aug 12	Aug 15	Aug 16	Aug 22
Hemoglobin (per cent)	68	64	80	79	
Red blood cells	4 3	4 15	4 4	4 5	5 5
White blood cells	950	1,575	1,400	1,200	3,100
Platelets	300,000	300,000	260,000	310,000	240,000
Polys nonsegmented (per cent)					9
Polys segmented (per cent)	6				
Lymphocytes (per cent)	88	98	99	99	62
Monocytes (per cent)	4	2	1	1	11
Plasma cells (per cent)	2				

Bone Marrow Studies

	Aug 11	Aug 14	Aug 22
White blood cells	50,000	45,000	175,000
Megakaryocytes	66	44	44
Mycloblasts (per cent)	2 0		2 0
Promyelocytes (per cent)		0 5	12 7
Mycocytes neut (per cent)			55 0
Mycocytes eos (per cent)			0 7
Nonsegmented (per cent)			10 0
Polys neut (per cent)	0 4		1 0
Lymphocytes (per cent)	17 6	3 5	2 0
Hematogones (per cent)	6 8	7 0	2 3
Plasma cells (per cent)	2 0	5 5	3 0
Reticulum cells (per cent)	0 8	2 0	1 0
Erythroblasts (per cent)	0 8	4 5	0 3
Normoblasts (per cent)	69 2	76 0	10 0
Megaloblasts (per cent)		0 5	
Megakaryocytes (per cent)	0 4	0 5	

DISCUSSION

It is important to note that thiouracil had been administered in each case for at least five to six weeks before symptoms of agranulocytosis developed. In the first patient, although the amount of the drug was reduced after the fourth week, severe agranulocytosis nevertheless ensued. Treatment was of no avail, and the patient succumbed to overwhelming toxemia, death taking place forty-eight hours after admission to the hospital and five days after the onset of the symptoms. In case 2 the drug had been given for six weeks, discontinued, and later given for one week. This patient was in poor physical condition and died of cardiac failure. The effectiveness of penicillin could therefore not be judged in this case. Case 3 was somewhat similar in that the patient died of a toxic hepatitis before marrow regeneration took place. Case 4 was almost moribund on admission. In the remaining five cases recovery took place following penicillin therapy, although their condition on admission appeared just as desperate as that of the four fatal cases. In these cases, penicillin therapy was started immediately upon the diagnosis of agranulocytosis.

Penicillin appears to be the ideal drug to combat the toxemia and the bacterial invasion that take place with agranulocytosis following the use of thiouracil, sulfonamide, or other drugs. In three of our patients, one or more of the sulfonamides had been given before admission to the hospital, so that the possibility of a sensitivity reaction was present and therefore the administration of all such drugs was considered contraindicated. While this paper was in preparation we had the opportunity to observe a patient, a physician, who developed agranulocytosis following the use of aminopyrine and cinchophen, sulfadiazine and sulfamerazine. He was given penicillin therapy and made a rapid and uneventful recovery.

The question arises with thiouracil, as it does with other drug therapy in which agranulocytosis takes place, as to why some patients develop sensitivity to the drug and others do not. It has been known that a large percentage of patients with subacute bacterial endocarditis develop terminal agranulocytosis as a result of sulfonamide therapy over a considerable period of time. Astwood suggests that the large dose of thiouracil given in his patient (2 grams daily for eight days) may have contributed to the development of the agranulocytosis. This would suggest, as in our cases of *subacute bacterial endocarditis*, that the cumulative effect of the drug may cause the development of the sensitivity and the subsequent toxic action on the bone marrow. It has been shown by Jones⁹ and by Tyson¹⁰ that the marrow in hyperthyroid states is hyperplastic, with marked activity of all the elements. It is therefore unlikely that these patients had any decreased marrow cellularity before the treatment was started. In case 6, for example, sternal marrow studies were made at the beginning of the therapy and presented marked activity, with a total nucleated cell count of 540,000 per cubic mm. This, according to our normal findings (Vogel, Erf, and Rosenthal),¹¹ showed increased activity. With the onset of agranulocytosis the first bone marrow count was 70,000 per cubic mm, which in a normal person would be considered only slightly low, but in this case represented only about one seventh of the original count. Examination of the sternal marrow after recovery revealed a total nucleated count of 475,000 per cubic mm and indicated a return to the normal cell count. In addition, the presence of a high percentage of monocytes in the peripheral blood on the second day (28 per cent) indicated a good prognosis, although the total peripheral white count was only 950 per cubic mm. This was reflected in the marrow findings of 61.6 per cent promyelocytes on the same day. The significance of monocytosis in the peripheral blood as a good prognostic sign in agranulocytosis has been emphasized previously by Rosenthal and Abel.¹² In case 5 the total nucleated count of the sternal marrow on admission to the hospital was 15,000 per cubic mm. After recovery it was 110,000. Thus the marrow cellularity also fell to approximately one seventh of its normal level. The effect of thiouracil on the marrow in these eight cases, although variable, involves essentially the myeloid elements. The erythroid elements and platelets are usually spared. The myeloid cells at the height of the agranulocytosis may have almost entirely disappeared. Plasma cells and reticulum cells, the latter often vacuolated, became very conspicuous in the smears. More recently a case was described by Newcomb and Deane¹³ in which thiourea given over a period of five weeks caused granulocytopenia and, in addition, marked

thrombocytopenia. The marrow findings are not mentioned. In all our cases the platelets were relatively undisturbed, although some showed a slight tendency to thrombocytopenia. The marrow smears in some showed a relative increase in megakaryocytes. In others the entire marrow may be depleted, with the nucleated red cells and megakaryocytes affected, as seen in a hypoplastic bone marrow. In cases 1 and 2 the nucleated red cells were not appreciably affected, but the myeloid elements were replaced by reticulum cells, hematogones, lymphocytes, and plasma cells. The latter were increased in the peripheral blood in the beginning of the recovery stage in case 5. The presence of myelocytes in the marrow in normal or increased percentage offers the best prognosis in agranulocytosis and may precede an increase in monocytes by one or two days (cases 6 and 7). In cases 8 and 9 the marrow showed a relative increase in lymphocytes and nucleated red cells. In case 8 the hemoglobin, red cell count, and platelets dropped over a period of days, indicating that the drug might affect these elements at a later period. It should be realized that while the blood and marrow may show hematologic improvement, the patient may nevertheless succumb to an overwhelming toxemia and hepatic damage.

In case 9 penicillin was given before any clinical symptoms were evident, although hematologic evidence of agranulocytosis was present. The patient had no fever, ulcerations, or any evidence of toxemia. Four days later the temperature rose, the pharynx became reddened, and ulcerations developed in spite of an increase in the amount of penicillin administered (20,000 units every three hours). In fact, the patient developed a peritonsillar abscess which had to be evacuated during the height of the agranulocytosis. Culture of this yielded only *B. coli*, which is penicillin-resistant. This would indicate that the drug cannot prevent all bacterial growth. At present larger doses of penicillin, 30,000 units or more every three hours, would seem advisable.

There has been an increasing number of reports in the past year on the use of thiouracil in thyrotoxicosis. The number of patients so treated has varied from 32 to as many as 174 patients in a recent report by Williams and Clute.¹⁴ Even in as large a series as the latter authors studied, only two patients developed agranulocytosis, both of whom recovered after several days of serious illness. In other reports¹⁵⁻¹⁶ this complication is mentioned as a serious consideration, but most of the authors did not encounter this condition themselves. Because of the small number of cases of agranulocytosis reported, many physicians feel relatively secure in treating their patients with thiouracil.

While there is no question that under certain conditions thiouracil is an excellent drug to tide a patient over an acute episode of thyrotoxicosis, and especially in preparation for thyroidectomy, its effectiveness is present only during its administration. It cannot be considered as a medical cure, since it does not affect the basic difficulty of thyrotoxicosis. Furthermore, from our series of cases it would seem more dangerous than survey of the literature would lead one to suppose. At our hospital fifty-four cases have been treated with thiouracil, and of this series six cases of agranulocytosis have developed. This is approximately 11 per cent. This does not include cases with mild or moderate leukopenia or with other complications. The remaining patients reported in our series were treated by

others and referred to us during the stage of agranulocytosis. Our percentage of agranulocytosis is therefore much higher than that from other groups. However, even excluding the patients who were referred to us in a state of agranulocytosis, our incidence of this serious complication is still above that of any other reported series. We cannot account for this discrepancy, since the dosage and the follow-up seem comparable. One can predict that in the future there will be an increasing number of cases of agranulocytosis, since additional courses of this drug will be given as recurrence takes place and there will thus be an opportunity for more patients to become sensitized to the drug. For this reason it would seem advisable at present to limit the use of the drug only to selected preoperative cases and those in which operation is contraindicated.

SUMMARY

Thiouracil has been found to be an effective drug in the treatment of hyperthyroidism. Agranulocytosis following its use occurred in nine cases, four of which terminated fatally. In five others a complete and rapid recovery took place following penicillin therapy. The latter drug is believed to be ideal for all cases of agranulocytosis, and especially those in which chemotherapy has been used and may have been responsible for the condition. Thus far we have not seen any report of any untoward effect on the hemopoietic system from the use of penicillin.

The use of antibacterial agents for the treatment of agranulocytosis was suggested by Dameshek and Wolfson²¹ in 1942. It was believed by these authors that patients with agranulocytosis died not of the leukopenia per se but of the sepsis which developed secondarily to the lack of granulocytes. Two very severe cases of aminopyrine agranulocytosis treated with sulfathiazole made complete recoveries. For the treatment of sulfonamide agranulocytosis, it was suggested that a preparation differing from that which had already been used be given. With the discovery of penicillin, and its complete lack of possible deleterious effect on the bone marrow, its use was suggested by Dameshek¹⁷ (1944). A report on the beneficial effects of this medication in a case of sulfonamide agranulocytosis was later reported by Dameshek and Knowlton¹⁸ and similar cases by Sprague and Ferguson¹⁹ and by Meredith and Fink.²⁰

Since sulfonamides may cause further toxic effect on the bone marrow, we feel that their use should be avoided in the treatment of agranulocytosis, especially where a history of previous use is obtained. We do not agree with others^{21, 22} who continue the use of sulfonamides in the treatment of leukopenia or agranulocytosis where these very drugs may have been responsible for the condition. It would seem better judgment to use penicillin, which by combating the bacterial invasion of the body and the consequent toxemia enables the patient to survive until the bone marrow cells regenerate.

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DIFFERENTIATION OF PERNICIOUS ANEMIA AND CERTAIN OTHER MACROCYTIC ANEMIAS BY THE DISTRIBUTION OF RED BLOOD CELL DIAMETERS

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IT is often difficult to distinguish between Addisonian pernicious anemia and other macrocytic anemias that will not respond to the specific materials potent in pernicious anemia. The purpose of this paper is to demonstrate some of the findings in a study of the distribution of red blood cell diameters that seem to be of help in making such a differentiation. Demonstrable differences of cell diameters in various kinds of macrocytic anemia will suggest fundamental differences of etiology and hence of treatment in such cases.

Mogensen¹ has commented upon differences in the distribution of red blood cell diameters in macrocytic anemias, comparing pernicious anemia with anemias refractory to liver therapy. Such macrocytic anemias have been discussed as cases of refractory anemia by Rhoads and Barker² and Bomford and Rhoads.³ A certain type of refractory macrocytic anemia has been designated achrestic anemia by Israels and Wilkinson.⁴ Rhoads and Barker divided refractory anemia into primary and secondary groups. In the primary group, the anemia seemed to exist independently of any other pathologic process. In the secondary group, the anemia depended upon a recognizable cause, such as Hodgkin's disease, degenerative disease of the liver, tuberculosis of the bone marrow, or the like. Bomford and Rhoads classified the bone marrows of refractory anemias into four major groups according to distinctive histologic characteristics. It is often difficult clinically to separate these cases of refractory macrocytic anemia from those of pernicious anemia until a proper trial with a liver preparation has been made. Pathologic processes upon which the manifestations of the secondary group depend may remain hidden during life. The clinical features may resemble closely those of pernicious anemia, including glossitis and achlorhydria. The histologic character of the bone marrow is often inconsistent with the blood findings, being aplastic, hypoplastic, hyperplastic, or sclerotic.

One feature that is apparently of value in distinguishing refractory macrocytic anemia from pernicious anemia is the Price-Jones curve. In the former condition there appears to be frequently a smaller degree of anisocytosis of the red blood cells at a given red blood cell level. In order to confirm this impression, the diameters of the red blood cells in a group of cases with macrocytic anemia were measured by the Price-Jones method.⁵ The cases studied included 35 of pernicious anemia, 17 of refractory macrocytic anemia, 33 of macrocytic anemia secondary to a variety of causes, and 10 normal individuals (table 1). The cases which were not pernicious anemia were selected particularly with care (1) that the

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TABLE I—Summary of Observations on the Blood*

Case No	R.B.C. Million per Cu Mm	Hb (%)†	Mean Cell Vol (Cu μ)	Mean Cell Diam (μ)	S.D. of Diam (μ)	Coeff of Var of Diam (%)	Notes
PERNICIOUS ANEMIA							
1	0.41	7	105.0	8.51	1.38	16.2	
2	0.44	22	140.3	8.74	1.07	12.2	
3	0.89	26	133.7	7.77	1.13	14.5	
4	1.02	74	109.8	7.90	1.20	15.2	
5	1.16	24	125.0	8.10	1.01	12.5	
6	1.32	32	139.4	8.60	1.10	12.8	
7	1.32	32	129.3	8.21	1.44	17.5	
8	1.38	33	118.8	7.67	1.03	13.4	
9	1.44	34	112.3	8.21	1.12	13.6	
10	1.45	34	124.1	7.80	0.95	12.2	
11	1.50	37	109.9	7.57	0.87	11.4	
12	1.54	43	143.3	8.47	1.32	15.6	
13	1.64	36	112.2	7.67	0.95	12.4	
14	1.74	53	151.7	9.10	1.50	16.5	Splenectomy 11 years before
15	2.25	50	108.0	8.36	1.00	12.0	
16	2.48	50	131.5	8.39	0.76	9.1	
17	2.64	54	114.4	7.84	0.77	9.8	
18	2.73	64	109.3	8.29	0.70	8.4	
19	3.00	65	114.0	8.46	0.76	9.0	
20	3.01	86	124.9	8.06	0.68	7.7	
21	3.04	71	115.0	8.24	0.76	9.2	
22	3.19	78	116.4	8.17	0.61	7.5	
23	3.23	69	86.7	8.10	0.68	8.4	Diagnosed 8 mo later when R.B.C. 1,760,000 per cu mm
24	3.33	63	108.0	8.35	0.64	7.7	
25	3.36	76	108.2	8.18	0.62	7.5	In partial remission
26	3.36	76	109.9	8.14	0.63	7.7	In partial remission
27	3.57	64	89.1	7.64	0.56	7.3	Iron deficiency also
28	3.70	87	102.3	7.94	0.68	8.5	
29	3.95	96	101.3	8.14	0.64	7.8	Remission for 6 years without treatment
30	4.14	85	104.2	8.45	0.60	7.1	In remission
31	4.19	94	102.7	8.30	0.69	8.3	In remission
32	4.51	84	96.0	7.47	0.57	7.6	
33	4.62	95	96.8	7.99	0.61	7.6	In remission
34	4.76	88	85.3	7.71	0.52	6.7	In remission
35	5.09	101	92.7	7.60	0.49	6.4	In remission
REFRACTORY MACROCYTIC ANEMIA							
36	0.69	21	133.5	8.35	0.76	9.1	Bone marrow aplastic at autopsy
37	0.71	16	118.3	7.15	0.94	13.2	Bone marrow hyperplastic at autopsy
38	0.92	14	96.9	8.10	0.79	9.7	Bone marrow hyperplastic at autopsy
39	0.93	20	104.0	7.56	0.83	11.0	Bone marrow aplastic at autopsy
40	0.95	26	126.9	8.25	0.89	10.7	
41	1.03	23	104.9	7.29	0.66	9.1	Bone marrow aplastic at autopsy
42	1.08	22	100.0	7.33	0.78	10.6	Benzol poisoning
43	1.40	30	123.6	7.99	0.76	9.5	Bone marrow hyperplastic by sternal biopsy
44	1.48	34	08.6	7.73	0.71	9.2	Bone marrow hyperplastic by sternal biopsy
45	1.53	74	115.4	8.04	0.82	10.2	Benzol poisoning
46	1.99	42	100.0	7.62	0.84	11.0	Bone marrow aplastic at autopsy
47	2.06	42	123.0	8.47	0.81	9.6	Bone marrow hyperplastic by sternal biopsy
48	2.21	48	117.0	7.34	0.73	10.0	Bone marrow aplastic at autopsy
49	2.23	53	100.0	5.4	0.80	10.6	
50	2.36	53	109.7	7.83	0.70	8.9	
51	2.42	40	111.5	8.01	0.61	7.6	Bone marrow hyperplastic by sternal biopsy
52	2.86	71	108.7	7.87	0.62	7.8	Bone marrow hyperplastic by sternal biopsy
ACUTE BLOOD LOSS							
53	0.94	20	124.7	8.20	0.92	11.2	Laceration of stomach
54	1.42	32	138.7	8.15	0.65	8.0	Cause unknown
55	1.86	45	110.0	7.71	0.62	8.1	Duodenal ulcer
56	1.73	39	101.1	7.64	0.70	9.1	Duodenal ulcer
57	2.16	34	90.2	7.73	0.82	10.6	Duodenal ulcer

TABLE 1—Continued

Case No	R B C Million per Cu Mm	Hb (%)†	Mean Cell Vol (Cu μ)	Mean Cell Diam (μ)	S D of Diam (μ)	Coeff of Var of Diam (%)	Notes
LIVER CIRRHOSIS							
58	2 90	70	117 9	8 46	0 62	7 3	
59	3 24	62	104 6	7 18	0 73	9 4	
60	3 37	82	121 0	8 02	0 67	8 4	
61	3 50	75	111 9	8 08	0 49	6 1	
62	3 50	74	104 8	7 95	0 65	8 1	
63	3 11	69	103 7	7 68	0 62	8 1	
HEMOLYTIC ANEMIA							
64	4 41	110	101 7	7 23	0 59	8 2	Chronic hemolytic jaundice
65	4 23	91	103 5	7 45	0 62	8 3	Chronic hemolytic jaundice
66	1 14	30	133 3	7 76	0 83	10 1	Reticulocytes 45 %
67	1 31	30	103 2	7 13	0 70	9 9	Acute hemolytic anemia following use of sulfanilamide
68	3 31	65		7 53	0 63	8 4	Malaria
69	1 74	42	129 3	8 23	0 68	8 3	Nocturnal paroxysmal hemoglobinuria*
70	1 81	40	118 0	8 87	0 81	9 2	Nocturnal paroxysmal hemoglobinuria
71	1 90	40	107 4	8 69	0 95	10 9	Nocturnal paroxysmal hemoglobinuria
72	2 25	48	117 8	8 21	0 92	11 2	Nocturnal paroxysmal hemoglobinuria
73	2 78	52	93 2	7 83	0 70	9 0	Nocturnal paroxysmal hemoglobinuria
LEUKEMIA							
74	0 99	22	109 1	7 42	0 79	10 6	Megakaryocytic leukemia
75	1 18	28	99 5	7 69	0 87	11 3	Aleukemic myelogenous leukemia
76	1 30	28	116 3	7 63	0 72	9 4	Acute monocytic leukemia
77	3 08	68	107 4	7 50	0 61	8 1	Acute myelogenous leukemia
MISCELLANEOUS CONDITIONS							
78	0 83	16	112 4	7 73	0 85	11 0	Myelosclerosis with myeloid metaplasia?
79	2 21	51	105 9	7 73	0 63	8 1	Hemolytic anemia with myeloid metaplasia reticulo- cytes 23 %
	0 82	20	138 8	7 81	0 93	11 9	Hemolytic anemia with myeloid metaplasia reticulo- cytes 38 %
80	4 03	91	108 9	7 57	0 56	7 5	Scurvy
81	3 51	75	100 4	7 57	0 64	8 5	Scurvy
82	3 02	70	118 4	7 96	0 63	7 9	Echinococcus cyst of liver
83	3 75	83	100 0	7 74	0 74	9 5	Arsenic poisoning psoriasis
84	5 91	120	101 7	7 50	0 64	8 6	Congenital heart disease
85	3 07	68	109 8	6 77	0 59	8 7	Uremia pyelonephritis
NORMAL							
86	4 56	90	91 0	7 35	0 49	6 7	
87	4 48	91	90 8	6 86	0 53	7 7	
88	4 42	86	96 8	7 15	0 50	6 9	
89	4 97	96	95 8	7 03	0 48	6 8	
90	5 00	99	92 8	6 88	0 49	7 2	
91	4 64	90	96 8	7 20	0 51	7 1	
92	5 13	100	90 9	7 14	0 46	6 4	
93	4 63	85	90 0	7 55	0 48	6 3	
94	5 66	113	91 6	7 35	0 53	7 2	
95	4 60	89	94 3	7 47	0 54	7 2	

* All patients were over 21 years of age. The patients with pernicious anemia all responded to liver. Gastric analysis in the cases of pernicious anemia showed no free hydrochloric acid in the group of refractory anemias free by drochloric acid was present in cases 38 39 41 44 46 48 49 51 and absent in cases 36 37 47 50 52

† Hemoglobin instrument is so standardized that 100 per cent = 15 Gm

anemia be macrocytic,* (2) that factors identifying it is quite different from pernicious anemia be clear, e g., as in hemolytic anemia, (3) that there be an absence of response of the blood to the administration of adequate amounts of liver extract. The cases of pernicious anemia were selected at random and all responded satisfactorily to liver extract. Most of these patients had received no liver or liver extract at the time the measurements of the red cells were first made, but a few, as noted in table 1, were receiving treatment and were in remission. No observations were made during the reticulocyte response to liver extract.

The standard deviation† of the red blood cell diameters is a convenient measure of the degree of anisocytosis. A certain variability in the diameters of red blood cells from normal persons is always present, but the standard deviation in the normal is usually not more than 0.55 micron. In severe anemia, the standard deviation of the red blood cell diameters may be as high as 1.5 microns when the red blood cells are less than 1,000,000 per cubic millimeter. The coefficient of variation‡ is also a measure of anisocytosis and shows values of from 6 to 8 per cent in normal persons to about 18 per cent in pernicious anemia. The figures for standard deviation and the coefficient of variation are given for the individual cases in table 1.

The Price-Jones technic of measuring the diameter of red blood cells by projection was used, with the exception that smears of capillary blood stained with Wright's stain were employed instead of venous blood and Jenner stain. Two diameters, maximum and minimum, of each of 500 cells were measured in every case. The red blood cell counts and hemoglobin values were obtained on venous blood. The hemoglobin was determined by the Sahli method, with apparatus calibrated so that 15.6 grams of hemoglobin or 100 per cent was equivalent to an oxygen capacity of 20.9 volumes per cent. The mean corpuscular volume was determined by the Wintrobe method.⁸

In figure 1, the standard deviation of the red blood cell diameters has been plotted as abscissa against the red cell count as ordinate for each case. The figure shows a reverse relationship between the standard deviation and the red cell count for the whole group of cases. When the red blood cell count was less than 2,500,000 per cubic millimeter, the standard deviation served to separate with fair distinctness cases of pernicious anemia from the other cases. At these levels all but 2 cases of pernicious anemia had standard deviations of more than 0.9 micron. The other cases of macrocytic anemia showed standard deviations of less than 0.9 micron except in five instances, in none of these was it more than 0.95 micron. In one of the cases of refractory anemia (case 37) the standard deviation was 0.94 micron at a red cell level of 710,000 per cubic millimeter. In one case of acute blood

* No case having a mean corpuscular volume of less than 100 cu. micra was included with the exception of only a few cases in which the mean corpuscular diameter was definitely increased. This excluded quite a large group of refractory normocytic or microcytic anemias in which the etiology was unknown. In 5 of the cases of pernicious anemia at high red cell levels the mean volumes were less than 100 cu. micra but the mean diameters were increased.

† The standard deviation is the measure in microns of the dispersion of the diameters; their range in size and the way in which the numerical frequencies of the different diameters are arranged.

‡ The coefficient of variation is the standard deviation expressed as a percentage of the mean and forms a measure of the variability that is independent of the unit in which the measurements have been made.

loss the standard deviation was 0.92 micron at a red cell level of 940,000 per cubic millimeter. Two cases of nocturnal paroxysmal hemoglobinuria⁶ showed standard deviations of 0.95 and 0.92 micron at red cell levels of 1,900,000 and 2,250,000 per cubic millimeter respectively. In one case of agnogenic myeloid metaplasia the standard deviation was 0.93 micron.⁷ The standard deviation of the red cell diameters in the miscellaneous group of patients was not significantly different from that for refractory anemia, but was considerably lower than that for pernicious anemia.

In cases with red blood cell counts that were greater than 2,500,000 per cubic millimeter, the standard deviation did not reliably distinguish pernicious anemia from the other types of macrocytic anemia.

In figure 1, it may be observed that the degree of anisocytosis varied with the degree of anemia. Average values for some of the observations in table 1 are given

TABLE 2.—*Different Types of Macrocytic Anemia: Average Values of Mean Cell Diameter and Standard Deviation at Varying Red Cell Levels*

Diagnosis	Red Blood Cells per Cubic Millimeter														
	up to 1,500,000			1,510,000-2,500,000			2,510,000-3,500,000			3,510,000-4,500,000			4,510,000-5,500,000		
	No Cases	Average Value M.C.D. (μ)	S.D. (μ)	No Cases	Average Value M.C.D. (μ)	S.D. (μ)	No Cases	Average Value M.C.D. (μ)	S.D. (μ)	No Cases	Average Value M.C.D. (μ)	S.D. (μ)	No Cases	Average Value M.C.D. (μ)	S.D. (μ)
Pernicious anemia	11	8.09	1.11	5	8.35	1.11	10	8.20	0.68	5	8.11	0.63	4	7.69	0.55
Refractory anemia	9	7.15	0.81	7	7.84	0.16	1	7.87	0.62						
Acute blood loss	2	8.17	0.78	3	7.70	0.71									
Liver cirrhosis							6	7.99	0.63						
Hemolytic anemia	2	7.44	0.11	4	8.49	0.84	2	7.68	0.66	2	7.34	0.60			
Normal condition													10	7.22	0.50

M.C.D. = mean cell diameter S.D. = standard deviation of diameter from the mean

in table 2, with consideration of the different red cell levels. These showed distinctly higher values for the standard deviation in cases of pernicious anemia at red cell levels of 2,500,000 or less.

Figure 2 shows drawings of red blood cells, traced by projection as in the Price-Jones technic, in two severely anemic patients, one having pernicious anemia, the other refractory anemia. Although the mean corpuscular volume and the mean corpuscular diameter in the 2 cases are approximately the same, the standard deviation in the case of pernicious anemia is much larger than in the case of refractory anemia. From the drawings of the red blood cells, this difference in degree of anisocytosis can readily be seen. The characteristic shapes of such curves have been commented upon by Price-Jones and by Mogensén. The curve for pernicious anemia at low red blood cell levels has a very broad base and shows skewness with the longer slope to the left. Such skewness signifies a relative predominance of

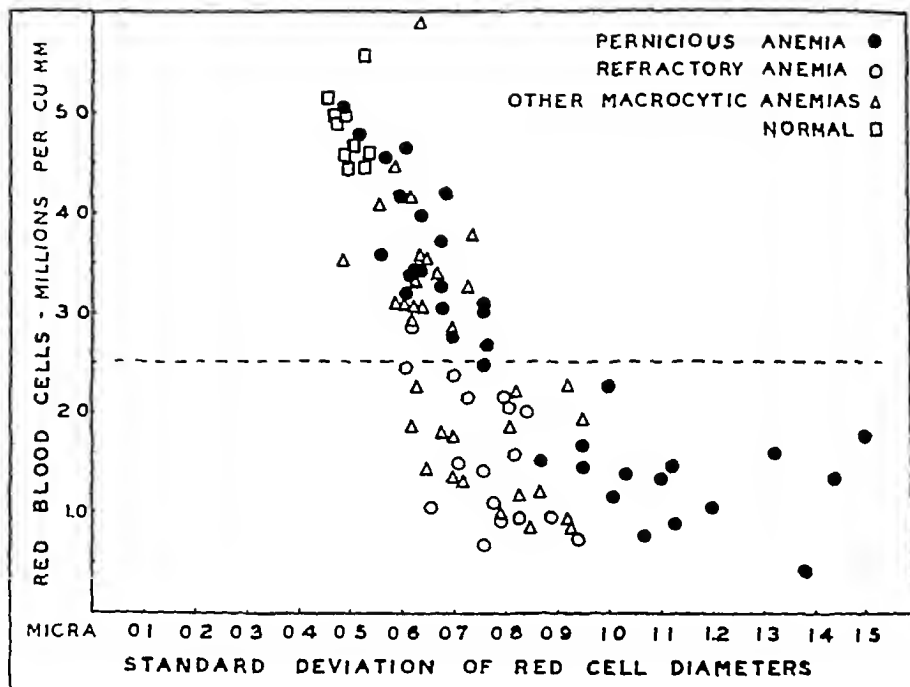


FIG 1 RED BLOOD CELL COUNT AND STANDARD DEVIATION OF RED CELL DIAMETERS IN DIFFERENT TYPES OF MACROCYTIC ANEMIA

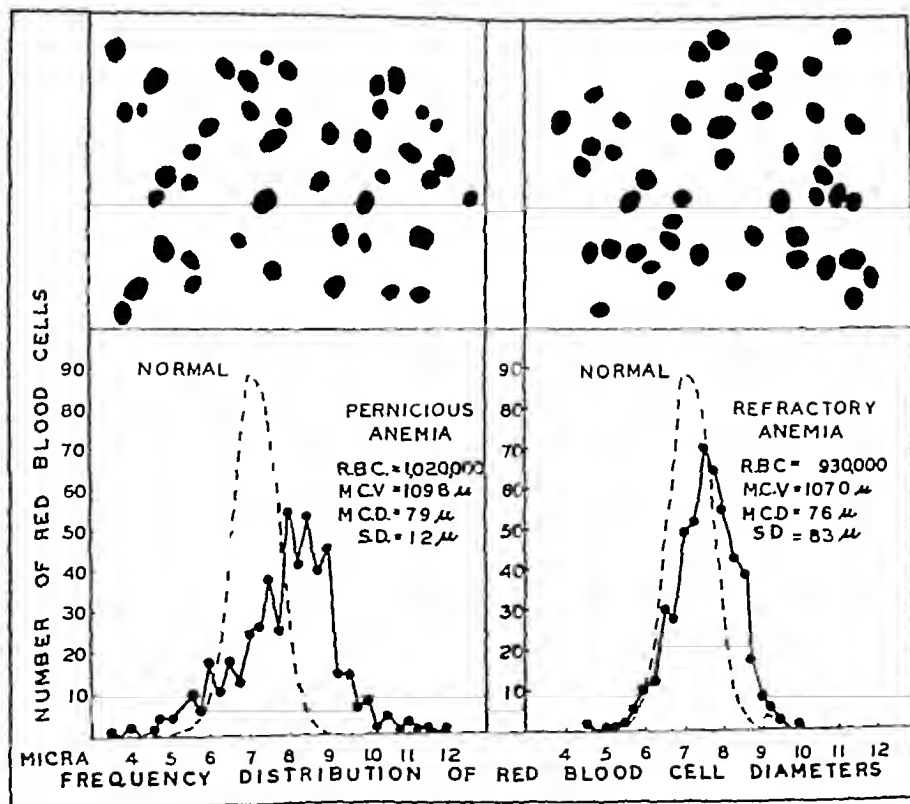


FIG 2 COMPARISON OF PRICE JONES CURVES AND SIZE AND SHAPE OF RED CELLS IN PERNICIOUS ANEMIA AND REFRACTORY ANEMIA

microcytes over macrocytes. At the higher levels, the Price-Jones curve tends to be symmetric, but occasionally there is slight skewness to the right. In a well treated patient the Price-Jones curve becomes normal.

Table 3 shows the frequency with which skewness to the left in the Price-Jones curve occurred at red blood cell levels of less than 3,000,000 per cubic millimeter in Addisonian pernicious anemia and the other cases of macrocytic anemia. Several methods of quantitating the degree of skewness may be employed—for example, determination of the mean-mode* difference, which will be zero in normal curves, negative in skewed curves with longer slope to the left, and positive in skewed curves with longer slope to the right. The mathematical analysis of skewness does not seem to be more advantageous than the results of simple inspection, which is sufficiently accurate for clinical purposes.

The important diagnostic information to be gained in studying the distribution curves of the red blood cell diameters in pernicious anemia appears to be derived from the following factors: (1) the degree of anisocytosis as revealed by the standard deviation or coefficient of variation, (2) the presence or absence of skewness of the curve with the longer slope to the left, and (3) the relation of these factors

TABLE 3—Presence or Absence of Skew Distribution Curves of Red Blood Cell Diameters in Different Types of Macrocytic Anemia with Red Blood Count of Less than 3,000,000 per Cubic Millimeter

Type of Anemia	No. Cases	Skewness					
		Slope to Left		None		Slope to Right	
		No. Cases	%	No. Cases	%	No. Cases	%
Refractory macrocytic	16	0	0	12	75	4	25
Pernicious	18	10	56	7	30	1	6
Miscellaneous macrocytic	20	—	10	1—	60	6	30

to the level of the red blood cell count. Other relationships have been studied, but require only brief mention. The standard deviation varies with the hemoglobin in the same manner as it does for the red blood cell count. In cases with less than about 40 per cent hemoglobin, the standard deviation serves as an aid in differentiating between pernicious anemia and the other macrocytic anemias. Mean diameter and mean volume of the red blood corpuscles at given levels do not distinguish pernicious anemia from the other macrocytic anemias. There is, moreover, only little relation between the mean diameter and the standard deviation of the red blood cell diameters in all the cases under consideration. The comparison of these two factors did not serve in any way to distinguish pernicious anemia from the other cases of macrocytic anemia. The same may be said in regard to comparison between the mean corpuscular volume and the standard deviation. The relative numbers of macrocytes and microcytes in a given preparation of blood, or the degree of anisocytosis, have been ascertained by various methods. These values provided no information of diagnostic help additional to that obtained through the use of the standard deviation.

* The arithmetic mean of the cell diameters is the average of the diameters as determined on 500 cells, expressed in micra.

Certain unusual types of macrocytic anemia responding to liver may be exceptions to the findings above. Those most commonly encountered in this country are the macrocytic anemia of pregnancy, sprue, and nutritional anemias, such as those associated with pellagra. On the other hand, such cases do not usually present the difficulties in diagnosis encountered between Addisonian pernicious anemia and certain macrocytic anemias refractory to specific therapy.

Three cases of macrocytic anemia accompanying pregnancy and responding to liver therapy have been studied by the present methods. The red blood cell levels and standard deviations of the red blood cell diameters in these cases were, respectively: case 1, R B C 860,000, standard deviation 0.77, case 2,* R B C 1,260,000, standard deviation 0.86, case 3, R B C 895,000, standard deviation 0.86. Here, it will be recognized, the anemia was severe, yet the degrees of anisocytosis as shown by standard deviations were relatively small. The Price-Jones curves in the first 2 cases were symmetric, in the third case, the curve was slightly skewed, with the long slope to the right.

Although macrocytic anemia is common in sprue, the literature suggests that the Price-Jones curves in this condition are often dissimilar in many respects to those in Addisonian pernicious anemia. Thus Fairley, Mackie, and Billimoria,¹⁰ who analyzed 67 cases of sprue, state: "Anisocytosis was the one outstanding feature especially as regarding increase in size. Microcytes were much less in evidence than the larger forms. Poikilocytosis and polychromasia occur, but to nothing like the degree observed in pernicious anemia." Examination of the Price-Jones curves of the cases reported by these authors reveals relatively symmetric curves with little tendency to skewness. The standard deviation was 0.95 micron or more in 5 of 7 cases in which the red cell count was 2,500,000 per cubic millimeter or less. Newham, Morris, and Manson-Bahr¹¹ report similar findings.

Little is known of the distribution of red blood cell diameters in other macrocytic anemias associated with nutritional deficiency. The anemia of nontropical sprue or of idiopathic steatorrhea is variable and often associated with iron deficiency. Rather marked increase of anisocytosis may be present, but the Price-Jones curves in the few cases reported¹ suggest certain differences from pernicious anemia, notably lack of the skewness with large left component.

CONCLUSIONS

1. Below the level of about 2,500,000 red blood cells per cubic millimeter, the degree of anisocytosis as revealed by the standard deviation and the coefficient of variation of the red cell diameters serves as a fairly accurate criterion for distinguishing Addisonian pernicious anemia from many other types of macrocytic anemia.

2. Below the level of 3,000,000 red blood cells per cubic millimeter, the asymmetric skewness of the distribution curve of the red cell diameters in pernicious anemia is an aid in distinguishing these cases from certain other types of macrocytic anemia.

* This case has been reported by Watson and Castle.⁹

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THE DIAGNOSIS OF HODGKIN'S DISEASE BY ASPIRATION BIOPSY

By LUCILE LOSEKE, M D , AND LLOYD F CRAVER, M D

BIOPSY by means of aspiration through a needle has become at Memorial Hospital an indispensable method of obtaining tissue for microscopic diagnosis. The technic is now well established, and the method has been repeatedly described.¹⁻⁵ Admittedly, skill in performing aspiration biopsy and in interpreting the tissue so obtained cannot readily be acquired except in centers in which considerable numbers of cases of neoplastic disease are treated, and in which the pathologists will take the trouble to familiarize themselves with the appearance of the material derived from various tumors by aspiration. It may be emphasized again that aspiration biopsy, as its name implies, is a method of aspiration of tissue through a hollow needle and is not a punch biopsy.

During the period of development of aspiration biopsy at Memorial Hospital, there was at first little or no attempt to apply it to the diagnosis of the lymphomatous tumors, as it was believed that it would seldom be reliable in that field, in which histologic diagnosis is so notoriously uncertain even with the benefit of having unimpeachable whole node sections. However, in recent years a number of cases has accumulated in which it has been shown that a diagnosis of lymphosarcoma or Hodgkin's disease can be reliably made on material obtained by aspiration. The immediate smear is seldom sufficient for a diagnosis of Hodgkin's disease. In nearly all cases the diagnosis rests on a paraffin section of the blood clot.

The purpose of this paper is to report the experience of five preceding years (1940-1944), and also in cases at present in the active files of Memorial Hospital, in establishing the diagnosis of Hodgkin's disease and related diseases by means of aspiration biopsy.

There are various conditions in Hodgkin's disease that may make it either impossible or undesirable to obtain conventional surgical lymph node biopsies. The patient may not present any significantly enlarged accessible peripheral nodes, while he may at the same time have an enlarged spleen or an infiltration in the lung. Enlarged peripheral nodes may be present, but forming one confluent mass, from which it would appear unwise to excise a wedge. The only accessible node may be in a location—e g, directly on the spinal accessory nerve—that renders excisional biopsy hazardous, or it may lie so close to the thyroid that it simulates thyroid cancer. One case not included in this series, because proof of diagnosis had previously been made by formal node biopsy, presented a periurethral mass, felt through the vaginal wall, that simulated urethral carcinoma. In one of the cases in this series a presternal tumor was at first thought to be a sternal chondroma.

Since a sound basis for rational treatment of neoplastic disease can be assured only by exact knowledge of its histology, the management of such cases as those just mentioned would be handicapped by much uncertainty on the part of the therapist, if it were not possible to obtain early in their course a proof of the

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diagnosis The so-called therapeutic test of diagnosis by irradiation is not to be trusted

METHOD

Aspiration biopsy is a method of aspirating tissue into the bore of a hollow needle As developed and practiced at Memorial Hospital, this method has certain orderly steps

- 1 The site within the tumor into which the point of the aspirating needle is to be inserted, is accurately localized

- 2 The overlying skin is prepared by shaving if necessary, and with iodine and alcohol

- 3 A wheal at the site in the overlying skin selected as the appropriate place for introduction of the needle, is anesthetized with 1 per cent novocain

- 4 A tiny incision is made clear through the skin with a no 11 Bard-Parker bistoury, so that elastic fibers of the skin will exert no drag on the needle

- 5 An 18-gage needle accurately fitted with a stilet, and having a rather short bevel, is carefully introduced through the incision in the anesthetized skin until the point of the needle reaches the desired site within the tumor

- 6 The stilet is removed and a 20 cc Record syringe is attached to the needle The piston is then drawn back so as to create a strong negative pressure within the needle-syringe system While this negative pressure is maintained, the needle is slightly advanced and withdrawn a few times, sometimes with rotation If negative pressure is partially lost by leakage, the syringe is detached and the air is expelled from it, then it is reattached and negative pressure is again produced

When soft tumors are being aspirated, from 0.5 to 3.0 cc of bloody material may be sucked into the syringe Firm or hard tumors may yield only a tiny bit of tissue, found within the bore of the needle Occasionally nothing significant will be obtained, and the procedure may have to be repeated, if possible

- 7 The pressure within the syringe is allowed to become nearly normal, so that when the needle is withdrawn there will be no spattering of blood and tissue inside the syringe, and the needle is then withdrawn

- 8 Any material collected within the syringe is allowed to clot if it will The clot is removed, placed on a small square of blotting paper, and then dropped into a small biopsy bottle containing 10 per cent formalin The clot is run through paraffin like any tissue biopsy, and sectioned

- 9 Material in the needle is pushed out by means of the stilet and blown onto slides, other fragments may be found inside the syringe walls or on the face of the piston This material is firmly crushed and smeared between slides and is quickly stained with hematoxylin and eosin This is known as the immediate smear

- 10 The procedure used when a tumor within the chest is aspirated does not differ essentially from that described above

Naturally great care must be used—first, in accurate localization of the tumor by means of straight postero-anterior and straight lateral roentgen films, second, in fluoroscopic check of the position of the point of the needle, third, in avoidance of trauma to the lung, fourth, in avoidance of any reflux or injection of tumor ma-

terial or air into the lung or into a blood vessel, fifth, in keeping the patient horizontal during the procedure, and very quiet for some hours afterward.

Currently at Memorial Hospital a considerably greater number of intrapulmonary malignant tumors are yielding microscopic diagnoses as a result of aspiration biopsy than by means of bronchoscopic biopsy, though the latter procedure is always given precedence unless it would obviously be useless.

MATERIAL

A tabular review of the method and site of biopsy in 242 cases of proved Hodgkin's disease, 121 of them seen in the years from 1940 through 1944, and 121 now in the active files of the hospital, is presented in table 1.

Of the 242 cases reviewed, open biopsy was the method used in 228, and cervical nodes were the type most often removed.

Aspiration biopsy established the diagnosis in 14 cases, or in about 1 case in 17. In addition to these 14 cases in which the diagnosis was made by aspiration biopsy,

TABLE 1—Types of Biopsies Performed

Year	Formal Surgical Biopsy			Miscellaneous	Aspiration Biopsy	Total
	Cervical Nodes	Axillary Nodes	Inguinal Nodes			
1940	25	6	1	1	3	36
1941	25	4	2	0	0	31
1942	23	3	0	0	0	26
1943	15	1	0	0	1	17
1944	5	3	1	0	2	11
Active	92	13	7	1	8	121
Total	185	30	11	2	14	242

* This group includes 1 case in which biopsy was obtained at laparotomy and 1 case in which thoracotomy was performed.

11 other cases were found in which for one reason or another aspiration biopsy had been tried first but had failed to yield satisfactory or sufficient material, and a subsequent formal biopsy had been required. Thus, of 25 cases in which for some reason aspiration biopsy was chosen as the first biopsy method, it succeeded in 14, or 56 per cent, and failed in 11, or 44 per cent. Failure of aspiration biopsy, unlike failure of a formal biopsy, does not mean leaving the patient with the scar of a useless operation.

Among the 11 cases in which aspiration biopsy was unsuccessful, the reports in 5 showed a finding of lymphoid tissue, in 2 the reports suggested tuberculosis or Hodgkin's disease, in 1 a suggestion of lymphosarcoma was made, in 2 Hodgkin's disease was suspected, but open biopsy to confirm the suggestion was requested, and in 1 an insufficient amount of material was reported. In some instances such reports have helped at least to make the examiner veer away from a previously considered diagnosis of carcinoma.

Among the 14 cases in which aspiration biopsy was successful in diagnosing

Hodgkin's disease, there were 9 in which the procedure was applied to enlarged peripheral nodes that were considered not easily amenable to open biopsy. The provisional diagnoses before aspiration biopsy in these 9 cases are shown in table 2.

In 1 case a presternal nodule, at first believed to be a chondroma, was punctured for aspiration.

The remaining 4 cases among the 14 with successful aspiration biopsies for Hodgkin's disease did not have accessible nodes or masses suitable for surgical biopsy. Two presented pulmonary infiltration suspected of representing bronchogenic carcinoma. In one of these, bronchoscopy was negative, and in the other the patient would not cooperate to permit bronchoscopy to be accomplished. In the third case the only finding was a mass projecting into the left lung field, in a patient with constitutional symptoms suggestive of Hodgkin's disease. In the fourth patient, there was no peripheral lymphadenopathy, but the liver and spleen were considerably enlarged and the symptoms suggested Hodgkin's disease. In this case splenic puncture was performed.

TABLE 2.—*Provisional Diagnoses in 9 Cases Presenting Enlarged Peripheral Nodes, in Which Aspiration Biopsy Proved the Presence of Hodgkin's Disease*

Provisional Diagnosis	No. Cases
Hodgkin's disease	1
Lymphoma (unspecified)	3
Lymphosarcoma	1
Tuberculous lymphadenitis	1
Metastatic carcinoma	2
Carcinoma of thyroid	1

The case histories of the 4 patients who had no peripheral nodes are presented herewith.

Case 1. H. M., male, a naval lieutenant, aged 29 years, was admitted Mar. 31, 1942.

The history included cough for three months, hemoptysis for one month, loss of from 20 to 25 pounds in two months. The examination showed infiltration in the anterior part of the left upper lobe of the lung, clinically consistent with tuberculosis, bronchogenic carcinoma, or Hodgkin's disease; there were no enlarged peripheral nodes. The sputum was negative. Bronchoscopy, performed Apr. 1, 1942, gave a negative finding.

In the aspiration biopsy of the lung, Apr. 8, 1942, the immediate smear yielded insufficient tissue; there were some cells suggesting carcinoma. The clot showed Hodgkin's disease (fig. 1).

Case 2. M. D., male, a longshoreman, aged 62 years, was admitted July 27, 1943.

The history showed productive cough for two years and increasingly severe. A diagnosis of tumor of the left lung was made by a local physician after roentgenography of the chest in April, 1943. Pleural effusion then developed, and 2 thoracenteses had been performed before admission. The patient had lost 14 pounds in three months. The examination revealed emaciation, moderate hepatomegaly, a left pleural effusion obscuring the lung, moderate normochromic anemia, and polymorphonuclear leukocytosis (14,200 white blood cells, 88 per cent polynuclears). There were enlarged peripheral nodes.

Thoracentesis was performed Sept. 4, 1943. Roentgen examination then disclosed rounded density in the right hilar region and fairly well defined density in the left hilum, extending into the parenchyma.

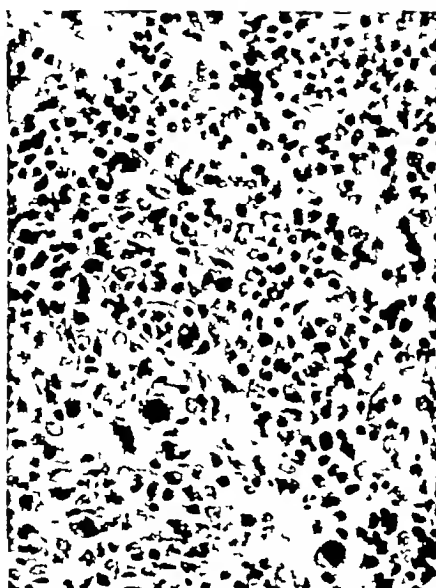
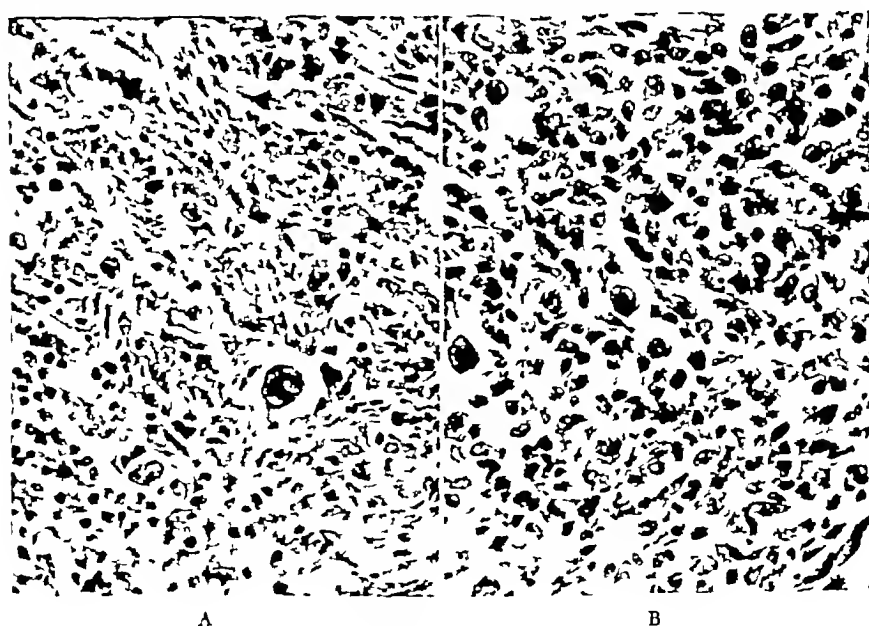


FIG. 1. CASE 1

Section of clot obtained from infiltrated portion of left upper lobe of lung by aspiration biopsy. Diagnosis of Hodgkin's disease made by means of this section confirmed clinically by subsequent course.



A

B

FIG. 2. CASE 4

- A. Aspiration biopsy from spleen before any nodes were available showing Hodgkin's disease.
 B. Section of surgical biopsy from axillary lymph node two months later confirming diagnosis obtained by spleen biopsy.

of the lung. Bronchoscopy was attempted but was unsuccessful because of poor cooperation on the part of the weak patient.

In the aspiration biopsy of the left lung, the smear showed lymphoid tissue. The clot showed Hodgkin's disease.

By Sept. 30, 1943, the spleen had become palpable, and a left infraclavicular node and axillary nodes had become significantly enlarged. The enlarged left infraclavicular node was then excised and its analysis confirmed the diagnosis of Hodgkin's disease.

Case 3. A. H., female, a typist, aged 40 years, was admitted Aug. 25, 1944.

According to the history, the onset of her illness had occurred ten months before, with severe upper respiratory infection followed by persistent cough productive of white sputum. Two months later, persistent general itching developed. Increased fatigability and dyspnea on exertion appeared shortly before admission. Examination showed a mass 9 cm. in diameter projecting from the left hilum into the lung and slight widening of the mediastinum. Moderate normochromic anemia and polymorphonuclear leukocytosis were found. No nodes were available for biopsy.

Aspiration biopsy of the left lung mass was made Sept. 7, 1945. The smear suggested carcinoma. The clot findings were consistent with those in Hodgkin's disease.

Irradiation as for Hodgkin's disease produced marked, prompt regression.

Case 4. S. L., male, a tomato packer, aged 56 years, was admitted May 29, 1944.

The history placed the onset of illness at two years before, with general severe itching. There had been a weight loss of from 35 to 40 pounds over two years, and night sweats and intermittent fever for one year. Dyspnea and wheezing had appeared. In the examination, widespread excoriation of the skin from scratching, emaciation, and an appearance of chronic illness were found. The spleen was enlarged to the level of the iliac crest. The liver was enlarged by two fingers breadth below the right costal border. There was roentgenographic evidence of some widening of the superior mediastinum and of hilar infiltration. Moderate anemia was present. No peripheral nodes were suitable for biopsy.

Aspiration biopsy of the spleen was made June 28, 1944. The smear gave evidence consistent with the criteria for Hodgkin's disease. The clot showed Hodgkin's disease.

On Aug. 28, 1944, an enlarged node was found in the left axilla. Formal biopsy of this node confirmed the diagnosis of Hodgkin's disease.

SUMMARY

1. Among 242 cases of histologically proved Hodgkin's disease treated at Memorial Hospital for the most part within the past five years, the diagnosis was established by open biopsy in 228, while in 14 cases aspiration biopsy yielded a reliable diagnosis.

2. In the 14 cases of Hodgkin's disease in which the diagnosis was made by examination of the sectioned clot obtained by aspiration biopsy, the material was from lymph nodes in 9 cases, from a presternal nodule in 1 case, from the lung in 3 cases, and from the spleen in 1 case.

3. Among the 228 cases in which formal biopsy was used to prove the diagnosis, aspiration biopsy had been previously attempted but had been unsuccessful in 11 cases.

Thus in 25 cases of the total number of 242, aspiration biopsy had been selected for good reason as the first method to try, and was diagnostically successful in 56 per cent of the group.

CONCLUSIONS

In cases of Hodgkin's disease without enlarged peripheral lymph nodes, yet presenting nodes or masses accessible to needle puncture, the method of aspiration

biopsy has often proved successful in establishing the diagnosis. Success depends in large measure on examination of a sectioned blood clot from the aspirated tissue by an experienced pathologist.

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EDITORIAL

THE ancients were fascinated by the blood. To it were ascribed many magical attributes. It became the focus of many ritual practices, the fundamental basis of racial differences, the crux of life itself.

With the invention of the microscope, two types of blood cells were at first revealed, and later a third—the platelet. Applying to the blood the aniline dyes newly developed in his time, Paul Ehrlich then transformed the research from the difficult art of peeping at fresh, unstained blood cells to one in which several kinds of beautifully colored objects could be gazed at unhurriedly. This allowed continued study and the separation of one cell from the other, because of this achievement alone, Ehrlich has often been considered the father of hematology. Study of the blood cells and their often minute variations proved fascinating, perhaps unduly so in some instances. In central Europe chiefly, there was so much preoccupation with the minutiae of cellular structure, transition forms, modes of cellular development, etc., that the forest could hardly be seen for the trees. Long-winded polemical articles—notably those by Pappenheim, who founded *Folia Haematologica*—made hematology a jousting ground in which almost fanatical wars were fought between monophyletists, dualists, and exponents of the polyphyletic school of blood cell development. The simple in blood became the complex, and for most physicians hematology became a dead subject, characterized by many Greek names and of only small practical importance.

The discovery, by the American investigators, Minot and Whipple, of the value of liver in pernicious anemia, came like a fresh breeze into a long-stagnant area. Not alone could a previously fatal disease now be quickly cured, but new yardsticks, such as the reticulocyte count, could be applied to assay the effects of therapy. Thus the dynamic or physiopathologic method of approach became in this instance as important as the purely histologic, and soon led to a host of investigations along physiologic, chemical, and biologic lines. As a result, the facts of the essential physiopathologic disturbances in pernicious anemia were worked out, together with the determination of the more fundamental importance of the disease as one of a group of deficiency syndromes. A quick transformation in hematologic thought soon became evident.

Hematology in central Europe lagged somewhat behind in this revolution, with much of the newer work coming to light in this country. Thus Naegeli, the late pope of hematology, reacted so violently to this American method for treating pernicious anemia that he conducted a poll of European hematologists regarding their reactions to it, and on the basis of this proved that liver had no value in the treatment of pernicious anemia.

The dynamic approach to studies of the blood and the blood-forming organs has led to many important discoveries. Among these—to cite but a few—are Castle's discovery of the lack of a proteolytic enzyme in the gastric juice of patients with pernicious anemia, the importance of an adequate amount of iron in the therapy of chronic iron deficiency states, the interrelationships between pernicious anemia,

certain vitamin B deficiency states, and sprue, the importance of vitamin K in hypoprothrombinemia, the concept of hemolytic anemia as an active process due to certain hemolyzing agents such as hemolysins, agglutinins, the spleen itself, etc., the Rh factor and its relationship to hemolytic disease of the newborn and to transfusion reactions, the importance of blood and blood substitutes in the therapy of shock, etc

One must confess however, that in some quarters the great emphasis laid upon the dynamic has led to almost complete neglect of the morphologic approach. I know of one laboratory, for example, where examination of a blood smear or of a sternal puncture preparation is looked upon almost disdainfully. This is reaction carried to the opposite extreme. The morphologic may give hints and even definite evidence of the physiologic: thus the spherocyte is indicative of a red cell that has been injured by a hemolytic agent, the orthochromatic macrocyte usually means a deficiency in liver extract principle, pancytopenia often indicates a bone marrow disturbance or a splenic dysfunction, etc. Those who wish to be well versed in the study of the blood should thus have a thorough knowledge of morphology as well as of the more modern concepts of dynamic medicine. This idea can perhaps be best exemplified in the well informed clinician. To be sure, in some quarters the hematologist is the hospital pathologist, in others he is the transfusionist, in still others, the clinical pathologist, the anatomist, etc. To our way of thinking, however, a thorough grounding in the field of internal medicine, together with a thorough knowledge of morphology and physiopathology, should make the complete hematologist. It is highly probable that no single method of looking at the blood is sufficient in itself, and that hematology is a broad field with morphologic, clinical, physiologic, biochemical, and immunologic relationships.

Studies dealing with the blood and the blood-forming organs have previously been published in numerous unrelated journals. To the difficulties of presenting such data in publications of a varied general range, there has been added in recent years the isolation or even literal extinction of leading European periodicals. A journal making current hematologic material systematically available through one central source has become an actual necessity. Such a journal in the Western Hemisphere will therefore provide not only a clearinghouse designed expressly to answer the needs of a major professional group, but also a single vehicle assuring continuity in reporting on advances as they are made. Like its name, *Blood*, it is hoped that the new journal will have universal interest, and thus perhaps serve as a small factor in fostering better international understanding.

WILLIAM DAMESHEK

ABSTRACTS

HEMATOPOIETIC TISSUES

OLIVER P. JONES, PH D

The Determination of the Formation of the Histiocytic Cells, The Role of Cholin M Chèvremont J Morphol 76 139-55, 1945

The importance of the role which histiocytes and macrophages play in the defense reaction of the organism cannot be overemphasized. There is ample evidence that in order to meet certain of these demands histiocytic cells may be derived from several sources viz lymphocytes fibrocytes connective tissue, and skeletal muscle. In this respect it is of interest to recall the original *in vivo* technic developed by Kolouch (Am J Path 15 413, 1939) for the simultaneous demonstration of histogenous and hemogenous cellular elements during the process of inflammation. In the present paper Chèvremont reported results from the observations of several thousand tissue cultures which indicate that cholin determines the transformation of various cells into the histiocytic state. He believes such a cytochemical reaction may occur under normal conditions as well as the pathologic conditions of contusion, inflammation and various degenerative processes. Leukocytes may indirectly favor the production of cholin by secreting an enzyme which can hydrolyze lecithin.—O P J

The Polymorphonuclear Cell and Its Occurrence Within the Larynx of the Cat B F Kingsbury Anat Rec 92 169-81, 1945

For quite a few years Bunting's views regarding the absence of neutrophil leukocytes from normal connective tissue—except bone marrow—have been accepted. The observations made by Kingsbury on a series of 12 fetal and newly born cats indicate contrary findings. He found areas of ectopic granulopoiesis in the aryepiglottic and thyroarytenoid folds, and suggests that such areas may perhaps be found in other mammals.—O P J

The Normal Myelogram in Albino Rats A M Endicott and M Ott Anat Rec 92 61-70, 1945

For the purpose of controlling investigations of certain experimental blood dyscrasias Endicott and Ott studied the peripheral blood and marrow obtained from 33 normal rats. Femoral marrow was found to be representative of the general marrow pattern throughout the body. These authors determined the amount of active marrow elements by means of a special projection apparatus and found it to be from 82 to 87 per cent. Smears of marrow cells were studied by diluting aspirated marrow with plasma (Endicott Stain Tech 20 25, 1945). The usual detailed classification of marrow cells was found to be neither desirable nor accurate, since the rates of nuclear and cytoplasmic maturation are not parallel. This observation is not unique for the rat since an asynchronism in the maturation of leukocytes is one of the outstanding defects in certain leukemias in man. Sundberg and Downey (Am J Anat 70 455 1942) also observed an asynchronism in the development of normal guinea pig marrow cells. In order to obviate some of these difficulties, Endicott and Ott used the following simplified classification of marrow cells, which has six categories viz (1) neutrophilic granulocytes—(a) older forms (metamyelocytes and segmenters) and (b) younger forms (promyelocytes and myelocytes), (2) eosinophilic granulocytes, (3) red cell series, (4) lymphocytes, (5) blasts, and (6) other cells. The myelogram for rats less than sixty days old is predominantly erythropoietic, whereas older rats have more granulocytopenia. This reversal in the myeloid-erythroid ratio takes place between the third and ninth weeks. These authors propose to use an index derived from the product of the mean percentage of cells and the amount of active marrow as a base line for future studies of hypo- and hypercellular marrows. This quantitative approach to marrow activity does not take into consideration cell size, mitotic activity, or the rate of delivery of marrow cells to the peripheral blood.—O P J

A Note on the Relatively High Number of Argentaffin Cells in the Mucosa of the Human Stomach
W. Sharpless Anat. Rec. 91: 37-42, 1945

Some years ago the theory regarding the site of formation of Castle's intrinsic factor was based on Meulengracht's experiments with hogs' gastric mucosa. Subsequent investigations on the argentaffin cell in pernicious anemia by Jacobson (*J. Path. Bact.* 49: 1, 1939) seemed to support this theory. However, Fox and Castle (*Am. J. M. Sc.* 18: 18, 1942) demonstrated the futility of applying ideas and concepts obtained from other animals to the human being. They showed that whereas the fundic portion of the hog stomach was not the source of intrinsic factor, the corresponding region of the human stomach had more of this factor than either the cardiac or the pyloric region. In the present paper, Sharpless used a slight modification of Bodian's protargol technique, which was originally used by Dawson for similar studies on laboratory animals. The results indicate that this new method is far superior to others. Sharpless found relatively large numbers of argentaffin cells in the fundic glands. Although the author did not mention it, this finding is very interesting, since, if these cells have anything to do with the elaboration of intrinsic factor, it agrees with the work of Fox and Castle and coincides with the area of degeneration seen in histologic preparations of stomachs from patients with pernicious anemia.—O. P. J.

ANEMIA

STEVEN O. SCHWARTZ, M.D.

JOSEPH F. ROSS, M.D.

Iron Deficiency Anaemia in Northwest Indian Soldiers *Martin Hynes, Mohammed Isbaj and T. L. Morris*
Brit. M. J. 1: 6-6-7, 1945

In the reported study approximately 1,400 native Indians were used. Two thirds of the men had less than 14 grams of hemoglobin per 100 cc., and the anemia was always hypochromic and either normocytic or microcytic. Following the administration of iron for two to three weeks, two thirds of the men having under 14 grams of hemoglobin and one half of the men having over 15 grams responded to iron therapy. Most of the moderate to severe anemias were associated with hookworm infestation. Dietary intake of iron was 60 mg., but only 1 mg. of this was derived from meat iron. It would appear that the non-meat iron was inadequately absorbed or utilized and that these men were living on the threshold of an iron deficiency, which was easily produced by the chronic blood loss due to the parasitism. The report helps emphasize the need for animal proteins and meat iron for normal hemopoiesis and especially hemopoiesis under constant strain.—S. O. S.

Progressive Addisonian Pernicious Anemia Successfully Treated with Intravenous Choline Chloride
F. B. Moosnick, E. M. Schlesher and W. E. Peterson J. Clin. Investigation 24: 78-82, 1945

This is a report of a 61-year-old white male with classical pernicious anemia who was well maintained on 3 cc. of crude liver extract for several years but became sensitive to liver extract when more purified preparations were used. In addition to developing a sensitivity, he also relapsed moderately. Because of his intolerance to liver he was given stomach preparations orally. During his period of relapse he developed catarrhal jaundice. Biopsy at this time revealed the marrow to be quite fatty, even though severely megaloblastic. Needle biopsy of the liver showed a fatty metamorphosis and the pattern of catarrhal jaundice. After a twelve-day observation period the patient was given 20 cc. of a 5 per cent solution of intravenous choline chloride daily for a period of sixteen days. This was followed by an increase in the red cell count and hemoglobin, an improvement in the general condition of the patient, a fall in the icterus index and a change to a less fatty normoblastic marrow. A 55 per cent reticulocyte count the third day also accompanied the therapy.

The authors suggest that the absence of choline from the purified liver extracts may possibly have to do with the lack of fat transportation and its increased deposition. They further suggest that since the improvement followed choline therapy without any additional liver extract or stomach extract, adequate amounts of the anti-anemic principle were stored but apparently due to the fatty liver were not elaborated, and finally that fat deposition beyond a certain volume percentage of the marrow may cause a disturbance in the utilization of the hematogenous substance elaborated by the liver. Unfortunately the

liver biopsy could not be repeated because of the patient's lack of cooperation. However, some time later, when the patient died due to an unrelated cause, the liver was found to be essentially normal.—S O S

Aplastic Anemia and Its Association with Hemochromatosis *Kurt Zelmacher and Margaret Bevans* *Arch Int Med* 75 395-403, 1945

The authors report on a 65 year old white male with a pseudo-aplastic anemia (hypercellular marrow with maturation arrest in the red cells and white cells, and a diminution of megakaryocytes) hemochromatosis and cirrhosis of the liver. They review 5 cases in the literature where hemochromatosis and aplastic anemia (4 pseudo-aplastic) coexisted. The authors believe that either intrinsic or extrinsic toxins are responsible for both aplastic anemia and liver cirrhosis. The previously damaged liver stores an increased amount of iron from absorption (intrinsic) and also from the breakdown products of the transfusions (extrinsic) and finally takes on the appearance of the organ in hemochromatosis. In cases of pseudo-aplastic anemia there is also increased hemolysis adding to the available intrinsic iron.—S O S

Nutritional Macrocytic Anemia and the Animal Protein of Diet *G F Taylor and P N Cbbuttant* *Brit M J* 1 800-02, 1945

This is a report of 50 consecutive unselected cases of anemia seen in a desert climate among adult male Indians of whom 1700 were meat eaters and 1180 vegetarians. Of the 50 cases 27 were lifelong vegetarians, 2 temporary vegetarians and 21 meat eaters. Hospital admission rate for anemia was 22 times greater among vegetarians than among meat eaters. Vegetarians mostly had macrocytic anemias (the so-called nutritional or tropical macrocytic anemias) microcytic or normocytic hypochromic anemias. No macrocytic anemia was seen among the meat eaters. The rations of both groups contained 3000 calories and over 80 grams of vegetable protein per day but the meat eaters had an additional 20 ounces of fresh mutton. The report definitely indicates that vegetable proteins are inferior to animal proteins as a source of protein for purposes of hemopoiesis.—S O S

The Artificial Production and Significance of Target Cells with Special Reference to Their Occurrence in Thalassemia (Cooley's Erythroblastic Anemia) *William N Valentine and J V Niel* *Am J M Sc* 209 741-52, 1945

Target cells were produced by the suspension of red cells in hypertonic serum or plasma made so either by the addition of chemicals or by evaporation *in vitro*. *In vivo* hypertonicity was achieved by the dehydration of dogs. Dehydrated animals showed a greater number of target cells than normal animals. Target cells could be made less prominent by resuspension in hypotonic plasma. Target cells are cells whose envelope is large in comparison to its contents (Barrett's original thesis). The authors suggest that a relationship exists between thalassemia and iron-deficient anemia, since both have an iron deficiency and target cells. Thalassemia, however, does not respond to iron therapy. The inherited inability to completely metabolize the iron to hemoglobin is suggested by the authors as a possible cause for the type of anemia seen in thalassemia.—S O S

Absorption of Ferrous and Ferric Radioactive Iron by Human Subjects and by Dogs *C V Moore, R Durbach, C Minnich, and H K Roberts* *J Clin Investigation* 23 755-67, 1944

Moore and his collaborators have attempted to settle the question of the relative therapeutic effectiveness of the two valence states of iron. Using the radioactive isotope of iron as a tracer substance they fed ferrous and ferric salts of iron to normal and iron-deficient human subjects and dogs and estimated the percent of iron absorbed from the amount of radioactive iron appearing in the circulating erythrocytes. Both ferrous and ferric salts were fed to each subject at different times in an attempt to control the experiments.

As previously reported by Hahn and his collaborators, the dogs absorbed both valence forms of iron equally well. A marked difference was observed in the human subjects however since they absorbed 1½ to 15 times more ferrous than ferric iron. These findings lend strong support to the clinical observations indicating the greater therapeutic value of ferrous iron, and re-emphasize the hazards of attempting to transfer to human physiology conclusions obtained from animal experimentation.—J F R

BLOOD GROUPS, TRANSFUSIONS, AND BLOOD SUBSTITUTES

JAMES M. BATY, M.D.

JOSEPH F. ROSS, M.D.

EUGENE L. LOZNER, M.D.

Role of Hemagglutinins Anti A and Anti B in Pathogenesis of Jaundice of the Newborn (Icterus Neonatorum Praecox) *Halbrecht I. Am J Dis Child 68 248-49 1944*

Halbrecht points out that all forms of icterus in the newborn, physiologic as well as pathologic are hemolytic in origin and that the liver does not participate in their causation. The mechanism of the production of erythroblastosis fetalis in its various forms has been explained. The genesis of physiologic icterus and of milder forms of pathologic icterus, however, has not been explained.

The author found 9 cases of true erythroblastosis fetalis in a series of 10,000 births. In addition there were 60 cases with jaundice which were designated as icterus praecox. In these cases the jaundice appeared within twenty-four hours after birth and at times was no less intense than that in erythroblastosis fetalis. The infants with icterus praecox appeared well-nursed vigorously, and all recovered. There were no hemorrhagic manifestations and the liver and spleen were not enlarged. The blood of 12 of these infants was examined; only 3 showed a mild anemia and none an abnormal number of nucleated red blood cells. There was an increase in the bilirubin content of the cord blood in all of the 15 cases in which it was determined, an average of 1.75 mg. per cent.

The blood of 95 per cent of these 60 infants was incompatible with that of the mother, in that the serum of the mother agglutinated the red blood cells of the infant. A similar incompatibility was demonstrated in only 30 per cent of 160 infants with physiologic icterus and in 26.5 per cent of 2,000 infants without icterus. The blood groups were determined in 15 cases; in each instance the mother was blood group O and the infant either A or B.

The author believes that icterus praecox is caused by the passage of isohemagglutinins anti A and anti B from the mother to the child through the placenta and hemolysis of the infant's red cells.—J. M. B.

Anuria With Special Reference to Renal Failure in Blackwater Fever, Incompatible Transfusions and Crush Injuries *H. Fay, A. Altmann, H. D. Barnes, A. Kandi. Tr. Roy. Soc. Trop. Med. & Hyg. 36 197-338, 1943*

This is the most comprehensive review yet to appear dealing with the pathologic effects of hemoglobinemia and hemoglobinuria. Two hundred and eighteen references are cited. An attempt is made to explain the anuria and oliguria occurring in blackwater fever, falciparum malaria, incompatible blood transfusions and crush injuries on the basis of similar renal abnormalities. Examination of available evidence leads the authors to conclude that the azotemia developing in these syndromes is *not* due to blockage of the renal tubules with precipitated products of hemoglobin, but is of extrarenal origin. The oliguria and anuria are believed to be the results of disturbances in electrolyte balance, dehydration, diminished blood volume, decreased renal circulation, and decreased glomerular filtration. Tubular degeneration precedes blockage with hemoglobin casts in the majority of cases, and it is suggested that the casts are a *consequence* and not a cause of the tubular damage and anuria.

Hemoglobinemia and hemoglobinuria *per se* have little or no toxic effect on the kidney. The mechanism by which large hemoglobin molecules pass through the normal glomerular membrane is still obscure, but it is fairly well established that the tubular epithelial cells reabsorb hemoglobin from the glomerular filtrate and return it to the body's economy. This process of reabsorption may be injurious to the tubular epithelium, but dehydration and electrolyte imbalance are believed to be more important.

Therapy of these syndromes should be directed at restoring and maintaining a normal blood volume, normal renal blood flow, and normal electrolyte balance. The hypothesis that alkalinization is an effective method of preventing or relieving oliguria and anuria cannot be supported by recent experimental or clinical evidence, and the dangers of overalkalinization are such that it is unwise to force large quantities of base into these patients.—J. F. R.

Hemoglobin and Plasma Protein Their Relation to Internal Body Protein Metabolism *Miller, L L, Robscheit Robbins F S, and Whipple G H J Exper Med 81 405-2, 1945*

By the intraperitoneal injection of hemoglobin (laked red cells) or the intravenous injection of hemoglobin digests, Miller and associates have been able to maintain nitrogen equilibrium in the protein-fasting dog for several weeks. The nitrogen utilization from parenteral hemoglobin is not quite so efficient as from parenteral plasma. In the anemic and hypoproteinemic dog intraperitoneal hemoglobin will cause an increase in hemoglobin and red cell and plasma protein content of the blood. These results are extremely pertinent to the investigations by Strumia on the preparation of a safe intravenous globin, as they indicate that such a material will contribute to protein nutrition as well as supply a colloid for the maintenance of plasma volume.—E. L. L.

The Use of a Modified Globin from Human Erythrocytes as a Plasma Substitute Preliminary Report *Strumia, M M, Clorick F H, Blake, A D, and Kerr W G Am J M Sc 269 436-42 1945*

The problem of how to use most efficiently and advantageously the red cell residue from large scale plasma production has been a vexing challenge to investigators during the past five years. Transfusions of red cell suspensions, topical application of red cell paste and production of human peptone have but partially answered the problem. Two other possible solutions are currently under intensive investigation. One is the production of a hemoglobin preparation safe for intravenous use and the other the degradation of hemoglobin into a globin which would be nontoxic and effective as a plasma substitute. The latter approach is now reported upon in preliminary fashion by Strumia and his associates at Bryn Mawr Hospital. They believe they have achieved a safe and osmotically effective preparation of globin and have administered it intravenously to 105 human beings, the largest single dose being 57 Gm. and the largest total amount 192 Gm. There has been no evidence of antigenicity. Pyrogenicity has been a moderate problem but the chief difficulty is apparently a vasomotor effect from some of the preparations. Strumia's report is indeed stimulating and his subsequent reports as well as the experience of others with globin and with hemoglobin preparations are awaited with considerable interest.—E. L. L.

HEMOSTASIS AND HEMORRHAGIC DISEASES

LEANDRO M. TOCANTINS, M.D.

Studies on Increased Coagulability of the Blood *T. R. Waugh and D. W. Ruddick Canad. M. A. J. 51 11-17, 1944*

The authors believe it probable that in circulating blood heparin and thromboplastin are in a state of dynamic equilibrium, and that in individuals with hypercoagulable blood there may be an uncompensated increase in the thromboplastin. To test this out, the clotting time of successive samples of normal venous blood, to which increasing amounts of heparin were added, was measured and a curve plotted representing mean values for a group of men. Curves obtained from the study of the blood in various abnormal states were then compared with the standard curve. An accelerated coagulation was observed during uncomplicated bed rest, in acute inflammatory conditions following operations, and after severe hemorrhage.

The changes in coagulability demonstrated by this careful investigation are clear and striking. No adequate support, however, is found for the conclusion that hypercoagulability depends upon an increase in the amount of thromboplastin in the blood. The reduced effectiveness of heparin as an anticoagulant in hypercoagulable blood may be due as much to a diminution in the heparin cofactor (the existence of which is presumed by the authors) as to the postulated excess of thromboplastin. Though operations and severe hemorrhage may conceivably introduce thromboplastin in the circulating blood, it is difficult to understand how prolonged bed rest will accomplish a similar effect. The hypercoagulability of the blood one day after operative procedures cannot be attributed to a rise in the platelets, which is generally not manifested until after the fifth day postoperatively.—L. M. T.

Hereditary Hemorrhagic Telangiectasia, Analysis of Capillary Heredopathies *Karl Singer and Wm. Q. Wolfson New England J. Med. 230 637-42 1944*

The authors discuss three capillary heredopathies usually associated with hemorrhagic tendencies

namely, pseudohemophilia (von Willebrand's disease), hereditary familial purpura and hereditary hemorrhagic telangiectasia. Separation of these three syndromes is usually based on the response to certain tests (bleeding time, petechial reaction of the skin) and the presence or absence of telangiectasias. The authors stress that coexisting combinations of these various types in the same family may be observed. They report three patients in one family in which telangiectasias, increased bleeding tendency and markedly positive petechial reaction of the skin were found. The sex distribution of the defects was about equal. It is pointed out that though pure types of hemorrhagic disorders may sometimes be separated clinically, the existence of multiple defects in capillary function precludes the use of tables of differential diagnosis of these disorders in which only rigidly demarcated types are considered.

The physiologic analysis of these vascular defects is skillfully attempted. The concept of deficient capillary contractility proposed to account for disorders of bleeding such as von Willebrand's disease (pseudohemophilia) is open to question. Visual demonstrations of discontinuance of blood flow through intact or severed terminal capillaries are insufficient indication that the capillary has actually contracted. Collapse of the vessel due to a fall in capillary pressure or an increase in surrounding tissue pressure may accomplish the same result.—L. M. T.

Physiological Aspects of Human Genetics: Five Human Blood Characteristics. *H. H. Strandkor*. *Physiol. Rev.* 44:5-66, 1944.

The author considers certain aspects of the inheritance of the hemophilic trait. Hemophilia in man is inherited as a sex-linked recessive trait. This implies that the hemophilic gene (h) and its normal allele (H) have their common locus on the X-chromosome and that a single hemophilic gene (h) will produce the condition in the male, whereas in the female two (h h) are necessary for its expression. Although some pedigrees suggest incomplete recessiveness on the part of the hemophilic gene, most heterozygous females do not show the defect. Hence, it seems justifiable to conclude that the common form of hemophilia is not only sex-linked but completely recessive. The heterozygous females which show a condition similar to hemophilia may possess a variant of the common allele or present a clinical picture like hemophilia but due to other causes. A partial answer to the question of why so few females are hemophiles (if any are) when so many males are affected is to be found in the manner in which hemophilia is inherited. If, as Haldane says, the incidence of hemophilia for the population of London is about 1 to 10,000 among males, we should expect only one female among a hundred million to be homozygous (h h) and therefore hemophilic. Moreover, to produce a homozygous female it is necessary that her father be a hemophilic (h) and her mother homozygous (h h) or a carrier (H h). The tendency of hemophiles to die early makes these matings unlikely. In the face of the selection pressure which the hemophilic trait encounters, it is odd that the frequency of the hemophilic gene is maintained at as high a level as it is. Since most hemophilic males fail to leave offspring, the (h) gene should gradually be reduced in frequency. The nearly constant frequency for this gene, however, seemed to Haldane to suggest that mutations from (H) to (h) take place at the rate of one mutation per 50,000 individuals each generation.—L. M. T.

Further Studies of Platelet Reducing Substances in Splenic Extracts. *Eugene P. Cronkite*. *Ann. Int. Med.* 21:5-6, 1944.

Acetone extracts were prepared from the spleen of two patients with idiopathic thrombopenic purpura, one patient with thrombopenic purpura associated with tuberculosis of the spleen, one patient with chronic malignant neutropenia and thrombocytosis, and one normal control (traumatic injury of the spleen). Amounts of extract equivalent to 30-45 Gm. of spleen were injected intravenously into rabbits and observations made of the platelets, bleeding time (puncture of ear vein), clot retraction (blood obtained by heart puncture) and capillary fragility (petechial reaction of the skin after application of negative pressure). Significant reductions in the number of platelets were observed in all animals except those receiving the extract from the normal spleen. The greatest reduction was found with the extract of the spleen from the patient with neutropenia and thrombocytosis. The thrombopenia lasted in most instances about twenty hours, was sometimes accompanied by prolongation of the bleeding time and diminution in clot retractility (qualitative estimation). The changes in the petechial reaction were not marked. The values recorded for bleeding time in normal rabbits are few and considerably longer than those in the literature; they afford an uncertain basis whereby any prolongations may be considered significant. The fact that the most striking results were obtained from the spleen of the patient with

thrombocytosis lends itself to conflicting interpretations. This paper adds plausible evidence to those of a similar nature previously reported, the demonstration still lacks conclusiveness, however.—L. M. T.

LEUKEMIA AND LYMPHOMA

LLOYD F. CRAVER, M.D.

Observations on Over One Hundred Cases of Myelogenous and Lymphatic Leukemia. *Friedmann, A. B., and Meier, J. M. Radiology 44: 341-43, 1945*

This is a report on 105 cases seen at Kings County Hospital in the years 1939 to 1941.

Fifty-three cases of myelogenous leukemia included 11 acute cases with an average survival of 2.5 months after onset, ranging from 2 weeks to 9 months and 42 chronic cases. Of the patients with the chronic form 34 were dead, having had an average survival of 44.2 months, ranging from 8 months to 114 years.

Fifty-one cases of lymphatic leukemia included 5 acute cases, with an average survival of 4.4 months and 46 chronic cases (36 deaths with an average survival of 17.5 months).

The authors state that irradiation of the bones produces a more gradual drop in white cell count and a longer remission than when treatment is confined to spleen or lymph nodes.

Total body irradiation is used in the advanced generalized cases and in those which had previously been treated locally but had become widespread. A diagnosable leukemia is always a generalized disease; no doubt this means that total body irradiation is reserved for the late cases with multiple widespread gross lesions. The doses given intermittently at 150 cm. target skin distances are usually 35 r a treatment and rarely 50 r.—L. F. C.

Lymphomatoid Diseases Involving the Eye and Its Adnexa. *McGawie, John S. Arch. Ophth. 30: 179-93, 1943*

The author reviews 21 histologically verified cases, including 17 primary lymphomatoid tumors and 4 secondary tumors.

Subconjunctival lymphomas all have the same clinical appearance, irrespective of their histologic type and clinical course.

Two cases are cited which were at first regarded as the granulomatous so-called pseudotumor of the orbit, but in which some years later a generalized lymphosarcoma developed.

Despite the generally poor prognosis for patients with localized lymphomatous tumors in the region of the eye, the author believes they should be treated by radiation with the justified hope that some will be cured and not have generalized lymphosarcomatosis. Eleven of the 17 primary cases in this series remained localized.—L. F. C.

Roentgen Therapy in Diseases of the Blood-Forming Organs. *Isaacs, Raphael. Radiology 44: 58-63, 1945*

This is a preliminary report of an attempted correlation of effects of x-ray therapy in a series of 980 cases, including various types of leukemia, Hodgkin's disease, and lymphosarcoma. The therapy is said to represent the current practice of roentgenologists all over the country.

The author believes that considerable importance attaches to the intervals between individual doses. As an example he cites a case of chronic myelogenous leukemia for which at first a dose of 200 r was given every other day to a total of 1,600 r in sixteen days, resulting in no demonstrable remission; whereas when to the same patient a 200 r dose was given daily for three days and one additional dose of 200 r three days later, a remission of three months ensued. However, the only evidence of remission shown in this article is the level of the white cell count, and it may be noted that this count ranged from 157,000 to 239,000 cells per cubic millimeter before the first course of treatment, but had decreased to a range between 134,000 and 79,600 in the ten days following that treatment. It should further be noted that the second course of treatment, allegedly more effective, because given daily, followed the first, allegedly ineffective course, after an interval of only twelve days.

One would naturally conclude that the regression of three months was produced by the combined effects of both cycles of treatment.

Another statement is to the effect that a relapse may be instituted if a small dose of x-ray treatment is

given, or if it is given over an area where it affects much bone marrow directly. To illustrate this statement the author cites a case of chronic myelogenous leukemia in which the following sequence of events occurred:

1. Five small doses (35 to 60 r) over the chest, followed by relapse in four weeks.
2. Two treatments of 75 r over the spleen (not on consecutive days!) followed by relapse in four weeks.
3. Four treatments of 75 r to spleen in nine days, followed by relapse in seventeen days.
4. Fowler's solution gave a remission for eight months.
5. Severe cough led to giving 7 treatments of 50 to 100 r over the chest at three day intervals and there followed a complete relapse.

The author's comment is that this patient should have responded to adequate treatment with x rays as he did to Fowler's solution and that the results (relapses) were those of stimulation rather than depression.

Perhaps one would be justified in terming some of the earlier treatments in this case inadequate (assuming that the early clinical condition of the patient had been such as to warrant heavier doses) but there is today little if any evidence in the opinion of most radiologists and biophysicists to support a belief in a stimulating effect of x-ray treatment.

The author states that the basal metabolic rate is a good guide to the need for therapy when there are fairly mature cells in the blood and blood-forming organs, but that when many blasts are present the basal metabolic rate alone is not an adequate guide. It is the reviewer's opinion that the basal metabolic rate can largely be dispensed with in deciding about the treatment of leukemia by radiation and that in most cases decision must be based on a consideration of many other factors.

In conclusion, the author gives his impression that it takes a longer time to produce a remission when treatments are given on alternate days or at three day to one week intervals, and that remissions are shorter, than when the doses are given on consecutive days and discontinued when the white cell count falls to 60,000 to 80,000. Such a general statement raises the question of what would be done for the patient having troublesome symptoms who has never been treated and whose white cell count is only 30,000.

It is a common error to speak of a small dose of x-ray, as the radiation delivered by x-ray machines as a heterogeneous bundle of rays of different wave lengths. It should be referred to as x-rays.—L. F. C.

NEWS AND VIEWS

It is appropriate that the emergence of a new journal with universal appeal should coincide with the first year of peace after so many years of bloodshed. Germination of the idea of the journal took a long time, but once actual sprouting took place there was rapid development. The idea of an American journal in the field of disorders of the blood and blood-forming organs took hold with enthusiasm and with a surprising lack of negative reaction. The name was a little more difficult. Should it be called *American Journal of Hematology*, *The Journal of Hematology*, *Blood*, or some other title? The simple and forthright title *Blood* was suggested by a few and strongly urged by our Consulting Editor. At first glance, it seemed too striking, but the more one thought about it, the more right it seemed to be. *Hematology* seemed to denote more the strictly morphologic approach, whereas present day studies of the blood were often functional and had to do with the blood itself. A poll of the editors disclosed about an even number voting for *Blood* and for *The American Journal of Hematology*. The plunge to *Blood* was then taken, in line with such modern titles as *Hygeia*, etc. The subtitle *Journal of Hematology* was added as a compromise, American being left out because one of our foreign advisers strongly urged as little provincialism as possible.

About our Editors, it seems that two of our Associate Editors are deans of their respective medical schools. Dr. Charles A. Doan at the University of Ohio, and Dr. Roy R. Kracke at the University of Alabama. Dr. Maxwell M. Wintrobe is the Professor of Medicine and Chief of the Department in the newly reorganized University of Utah Medical School. Dr. Thomas Hale Ham, of the Thorndike Memorial Laboratory of Boston, is temporarily with the United States Army Chemical Warfare Division, where he has done an outstanding job. Dr. Nathan Rosenthal, of New York City, is one of the pioneers of hematology in this country. Our Consulting Editor, the Nobel Prize Laureate, Dr. George R. Minot, needs no introduction. The Assistant Editors are two young men, one from Boston, Dr. Joseph F. Ross, and the other from Chicago, Dr. Steven O. Schwartz, in both of whom the Editor has confidence, they will undoubtedly reduce some of the editorial burdens. The Advisory Editorial Board, although admittedly incomplete, contains men of note from various parts of the country and in special fields. Dr. James M. Bary, of Boston, is a pediatrician, and Dr. Lloyd Craver, of the Memorial Hospital in New York City, is a medical man, radiologist, and tumor specialist. Dr. Louis S. Goodman, Professor of Pharmacology at the University of Utah Medical School, has written one of the most outstanding medical texts of this generation. Dr. Russell L. Haden, of Cleveland, and Dr. Cyrus C. Sturgis, of Ann Arbor, Michigan, need no introduction to those reading this journal, both having been top-

flight men in their fields for years. Dr. Oliver P. Jones, of Buffalo, is our morphologist and has often expressed himself dynamically in what are usually considered static subjects. Dr. Philip Levine is well known for his work with the M and N, and Rh blood factors. Dr. Eugene L. Lozner, formerly of the Thorndike Laboratory in Boston, has done some outstanding work in blood plasma at the Naval Medical Research Institute in Bethesda, Maryland. Dr. Stacey A. Mettier has carried the torch of hematology to San Francisco. Dr. Carl V. Moore, of Washington University in St. Louis, Missouri, is an editor in his own right—editing the newly reorganized *Journal of Laboratory and Clinical Medicine*. He is a prime exponent of the physiologic approach to hematologic manifestations. Dr. F. H. L. Taylor, a biochemist, has for many years participated in numerous investigations at the Thorndike Memorial Laboratory along chemical lines. Dr. Leandro M. Tocantins has for many years been a mainstay at Jefferson, where he has carried on many investigations in blood-coagulating mechanisms. Dr. C. J. Watson, Professor of Medicine at the University of Minnesota, is a physiologico-biochemical internist whose brilliant work in the field of the porphyrins is not outdone by his professorial ability in teaching internal medicine. From Canada, we have Dr. R. F. Farquharson, of Toronto, who has made many contributions in the field of pernicious anemia.

Enlisting the aid of our Latin-American friends in this new venture has been of foremost importance. All have been enthusiastic, with the result that a group, as yet incomplete, of outstanding Contributing Editors from the various countries of the Western Hemisphere has been formed. These include Professor I. Gonzales-Guzman, Dean of the School of Medicine at the University of Mexico, Dr. Moises Chediak, of Havana, Cuba, Dr. Ramon Suarez, of Puerto Rico, Professor Alberto Hurtado, of Lima, Peru, Dr. Paul Canzani, of Uruguay, Dr. Alfredo Pavlovsky, of Buenos Aires, Dr. Jose Oria, of São Paulo, and Dr. W. O. Cruz, of Rio de Janeiro, Brazil. Within the next few months an official list of contributing editors from the Latin-American countries, as well as from Europe, South Africa, Australia, etc., will be announced. It is hoped that the new journal will be truly international in its scope.

A new organization, The New York Society for the Study of the Blood, has recently been formed. An unusually large group of charter members joined enthusiastically in the new venture. The first group of officers includes the following: President, Alexander S. Wiener, Vice-President, Paul Reznikoff, Secretary-Treasurer, Dr. Peter Vogel. The stated purpose of the Society is the furtherance of research and the dissemination of knowledge concerning the allied fields of hematology, blood grouping, and transfusions. Meetings are to be held thrice annually.

The American College of Surgeons, with the active participation of several pharmaceutical houses, has embarked on a large program of visual education

utilizing motion picture scenarios expressly made for the project. The Armour Company has taken on the hematologic assignment, and a series of six to eight films of forty-five to fifty minutes in length is in preparation. The Editor has seen and helped to criticize the first of these films, which deals with the Hemopoietic Principle. The project deserves wholehearted consideration from all those interested in the blood, since wide dissemination of knowledge of this allegedly difficult subject will be a very great service.

Newspaper reports from Japan indicating the development of blood cell reactions in certain individuals exposed to the effects of the atomic bombs are of great interest and may add another etiologic factor to the now long list of materials which cause involvement of the bone marrow. Benzol, chemicals related to benzol or containing the benzene ring, arsenic, gold, the sulfonamide drugs, etc., may all produce either total or selective involvement of the bone marrow. Total involvement results in pancytopenia, but selective involvement may cause either anemia, leukopenia and granulocytopenia, or thrombocytopenia. Combinations of these effects are also possible. X-rays, radium, radioactive materials such as thorium and mesothorium, and artificial radioactive chemicals such as radiophosphorus, etc., may also affect the bone marrow in much the same way. The atomic bomb, with its presumably great radioactivity, may have induced severe leukopenia and granulocytopenia in some individuals and thus have resulted in agranulocytosis with its attendant sepsis. It will be of interest to have some exact scientific data on these alleged effects.

One often wonders why the newspapers and the national press bureaus as well as the radio chains are so interested in leukemia. Any case of this disease, particularly in a child, which happens to become known to a newspaper, is immediately given a big play and is the occasion for much dramatics. Telephone calls are made to specialists all over the country, a search is undertaken for donors from cured cases, daily bulletins from the hospital are prominently displayed, etc. Unfortunately, none of this hubbub is ever productive of any results, except perhaps to raise false hopes in the minds of the hard-hit family.

Acute leukemia is a rapidly growing, proliferative process of one of the white cell series. The consensus of opinion indicates that it is a highly malignant tumor, which—like the blood cells in general—is generalized from its inception. Most of the effects of this rapid growth are on the marrow, which becomes overwhelmed by abnormal cells, as a result of which rapidly progressive anemia, granulocytopenia, and thrombocytopenia ensue. No way has as yet been discovered to reverse this apparently irreversible and irresistible phenomenon. X-rays, radiophosphorus, chemicals, transfusions, etc., offer little or no relief, and the course is relentlessly downhill. There may be recovered cases—we have been shown data on a few of these. One always wonders, however, whether the diagnosis in these was correct. The chances are very strongly in favor of a leukemoid reaction. In any event, until

radically new methods of therapy, or perhaps prevention, appear, it seems silly for the newspapers to continue their frank ballyhoo of this disease. It is hoped that modern investigators will before too long come up with an answer to the enigma of this tragic disease. Results of progress will undoubtedly be quickly disseminated.

It is hoped that our subscribers, and other readers, will feel free to submit notes of current hematologic interest, critical comment regarding the various articles, as well as other material for publication in this column of News and Views.

BOOK REVIEWS

Atlas of the Blood in Children By KENNETH D. BLACKFAN, LOUIS K. DIAMOND, AND C. MERRILL LESTER
The Commonwealth Fund, New York 1944 320 pages 70 plates in full color \$12.00

This *Atlas* of 320 pages consists of 158 page text of hematology and 70 lithographed plates in full color, all in a handsome volume of 7½ by 11 inches. The text is brief but it is usually adequate and is arranged in conformity with the illustrations. This arrangement which, in the consideration of anemia stresses the element of red cell size rather than that of etiology, brings together such odd bedfellows as hypoplastic anemia and hemophilia in both of which the red cells are frequently normocytic and normochromic. Although the determination of red cell size in anemia is often of both diagnostic and therapeutic consideration it should never be forgotten that etiology must always take precedence. For this reason the reviewer would perhaps disagree with the contention of the authors that by selection of the predominating type of cell in a stained film and by comparison with those plates presenting like features, the student may reach a correct diagnosis of the disease condition at hand. This is perhaps putting too great a stress on morphology, the emphasis in recent years having shifted toward pathologic physiology.

Since the *Atlas* is devoted to the blood in children there is naturally no discussion or illustration of Addisonian pernicious anemia nor of polycythemia vera. The section on erythroblastosis fetalis is unusually good, as are the several plates illustrating the condition. The plates are after all the *raison d'être* of the book. The blood cells are ordinarily reproduced in very large magnification ($\times 1500$), i.e. about one and one half times larger than one customarily sees them with the microscope. The illustrations are unusually clear and lifelike showing an excellent color value on the part of the physician artist. They are, in fact, so lifelike that some of the poorer smears (as in plate 60 Subacute Monocytic Leukemia) are faithfully copied with a resultant faded appearance: the monocytic granules being conspicuously lacking. The plate labeled Acute Monocytic Leukemia must be seriously questioned as an example of the disease: the cells looking more like early lymphocytes than they do monocytes. In fact, monocytes fare rather badly throughout, as in plates 47 and 48 (acute primary tuberculosis), although the monocytes in plate 50 are well depicted. The lymphocytes on the other hand are beautifully shown, particularly in infectious mononucleosis and the young and toxic polymorphonuclears of severe sepsis are nicely reproduced. There are a number of beautiful plates on Mediterranean anemia although the target cell or leptocyte is not emphasized. Likewise the plates of congenital hemolytic anemia fail to stress or to present good pictures of the spherocyte so characteristic of that disease, particularly in crisis.

With the present great interest in the bone marrow it is unfortunate that plates of the marrow could not have been presented in connection with those of the peripheral blood. Perhaps this is a task for a future edition.

The lithography as a rule is good although in some of the plates it tends to be rather muddy. One wonders too about the necessity for a yellowish rather than a white background for the cells. The book is an art work throughout, the print being unusually large and legible and the chapter headings unusually well designed. It serves a useful purpose in the study and teaching of hematology.

Medico-Legal Blood Group Determination Theory, Technique, Practice By DAVID HARLEY M.D., B.Sc. F.I.C.
The Laboratories of the Inoculation Department St. Mary's Hospital, London Pp. 119 New York
Grune & Stratton 1945 \$3.50

This is a short but rather complete monograph on the application of blood grouping principles to forensic medicine. The author devotes the first quarter of the book to a brief but very lucid explanation of the theory of the A-B-O and M-N blood groups, including sections on heredity and on extravascular occurrence of antigens A and B. The next portion of the volume is an explanation of the author's particular technic of performing the grouping tests, which is a glass slide method. Included also are details on examination of blood and secretion stains for antigens A and B and corresponding antibodies. In the last portion of the book detailed studies of a number of actual medico-legal cases are presented.

Dr Harley has produced an excellent short book for the beginner in medico-legal blood grouping. Perhaps its chief virtue is the omission of as yet unsubstantiated methods and concentration on accepted methods with the A-B-O and M-N groups. This volume makes excellent reading for workers in legal medicine as well as for those wishing simple elucidation of the theory behind such work. There is no discussion of the Rh factors.

Leukopenia and Agranulocytosis By WILLIAM DAMESHEK, M.D., Clinical Professor of Medicine, Tufts Medical School. Edited by HENRY A. CHRISTIAN, A.M., M.D., LL.D., Sc.D. (Hon.) F.A.C.P., Hon. F.R.C.P. (Can.), Hersey Professor of the Theory and Practice of Physics, Emeritus, Harvard University. Reprinted from *Oxford Loose Leaf Medicine* with the same page numbers as that work. New York: Oxford University Press, 1944. Price \$1.75.

This small volume (less than 100 pages) fulfills the promise of its preface: it is a compact but comprehensive treatise on leukopenia and agranulocytosis. Both conditions occur not infrequently as toxic manifestations of sulfonamide therapy. Knowledge and understanding of the subjects of this monograph are therefore of great importance for the practicing physician.

The classification of leukopenia, with particular reference to etiologic factors, is thorough and gives an excellent basis for the differential diagnosis of conditions causing a scarcity of leukocytes in the peripheral blood. It is out of the scope of the author's treatise to give a detailed account of each of the diseases causing leukopenia. Consequently, this section of the book carries but a few case reports to point up briefly the pertinent data for the physician whose background in hematology is not especially rich. The discussion of symptomatic splenic leukopenia is in greater detail and is particularly good.

A more condensed yet easily read study of agranulocytosis and a better review of the literature on the subject could scarcely be found. The physiopathologic mechanisms are presented so clearly that the steps in the evolution of the disorder can be thoroughly appreciated. Much consideration is given to therapy reported as far from satisfactory despite occasional short-lived use of some drug which claims to reduce mortality to 30 to 50 per cent. Dameshek states that the fatality in this disease is about 80 to 90 per cent and asserts that death is not due to granulocytopenia per se but to bacterial invasion in a body stripped of its normal leukocytic defense. Emphasis is placed therefore upon combating sepsis (with one of the sulfonamides, preferably sulfathiazole or penicillin) as the most important factor after discontinuance of the sensitizing drugs. Yet when faced with a disease of such violent intensity as agranulocytosis, Dameshek feels that one is justified in using any measure possibly offering a contribution to the recovery. These additional methods which he considers are x-ray therapy over the long bones, nucleic acid derivatives including pentose nucleotides and adenine sulfate, transfusions of blood, leukocytic cream, liver extract and foreign proteins.—*Reprinted with the permission of U.S. Naval Medical Bulletin*

SOME NEWER CONCEPTS OF THE NATURAL DERIVATIVES OF HEMOGLOBIN

- I GENERAL CONSIDERATIONS
- II THE SERUM BILIRUBIN AND BILIFUBINURIA
- III THE ERYTHROCYTE PROTOPORPHYRIN

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I GENERAL CONSIDERATIONS

PROTOPORPHYRIN is the underlying porphyrin of the hemoglobin molecule.¹ The name simply indicates that protoporphyrin is a representative or prototype of the various naturally occurring porphyrins insofar as its widespread occurrence and physiological significance are concerned. Protoporphyrin is the substance which binds the iron of the hemoglobin molecule. This was first established by the studies of Kammerer,² and of Fischer and Zeile,³ the earlier work of Hoppe-Seyler,⁴ Nencki and Sieber⁵ having indicated that the underlying porphyrin of the hemoglobin molecule was hematoporphyrin. The latter substance is formed when hemoglobin is treated with concentrated sulfuric acid, but, as Fischer and his associates have clearly shown, it is an artificial compound not occurring naturally. It is unfortunate that the term hematoporphyrin is still used in many textbooks of biochemistry and clinical chemistry in referring to one or another of the naturally occurring porphyrins.

In figure 1, the structural formula of the ferrous complex of protoporphyrin is shown. In accordance with the terminology of Anson and Mirsky⁶ this is perhaps best designated as heme. It is seen that the porphyrin ring is characterized by four pyrrol nuclei, each having a nitrogen at the apex of a ring of four carbon atoms. Each of the pyrrol nuclei is connected to one of its fellows by a methene (CH) bridge. The protoporphyrin molecule is distinguished from other porphyrins by the presence of two vinyl (CH=CH₂) groups, which are retained when the porphyrin ring opens as it does in the formation of bile pigment, as seen on the right in figure 1. The protoporphyrin molecule is a di-carboxylic acid, two carboxyl groups being present in the form of propionic acid rests. It is probable that the globin or protein fraction of the hemoglobin molecule is attached to these carboxyl groups (fig. 1). Globin constitutes 96 per cent of the hemoglobin molecule. Since the molecular weight of the heme is slightly more than 600, and that of globin 60,000 to 70,000, it is evident that if there were one molecule of heme for one of globin, the heme would constitute but 1 per cent of the hemoglobin molecule rather than 4 per cent as noted. This, together with additional evidence⁷, indicates an attachment of four heme molecules to one of globin.

It has been an important question in the past as to whether protoporphyrin is intermediate in the pathway between hemoglobin and the bile pigments. It was formerly believed, probably because of the ease with which hematin is formed in

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vitro by the treatment of hemoglobin with either acid or alkali, that hematin, a ferric complex of protoporphyrin, was the first step in the transition to bile pigment. Anson and Mirsky⁶ showed that hematin as formed naturally is still attached to globin. In recent years, Hamilton Fairley⁸ has shown that hematin is bound to some portion of the albumin fraction of the plasma protein when it is present in the circulating plasma in pathological states such as, for example, blackwater fever, severe liver disease, gas bacillus sepsis, and others. It is important to note that globin has approximately the same molecular weight and the same electrophoretic behavior as albumin, so that a hematin formed under natural conditions and still attached to globin would be expected to be associated with the albumin fraction. Fairley has employed the term methemalbumin to designate this as-

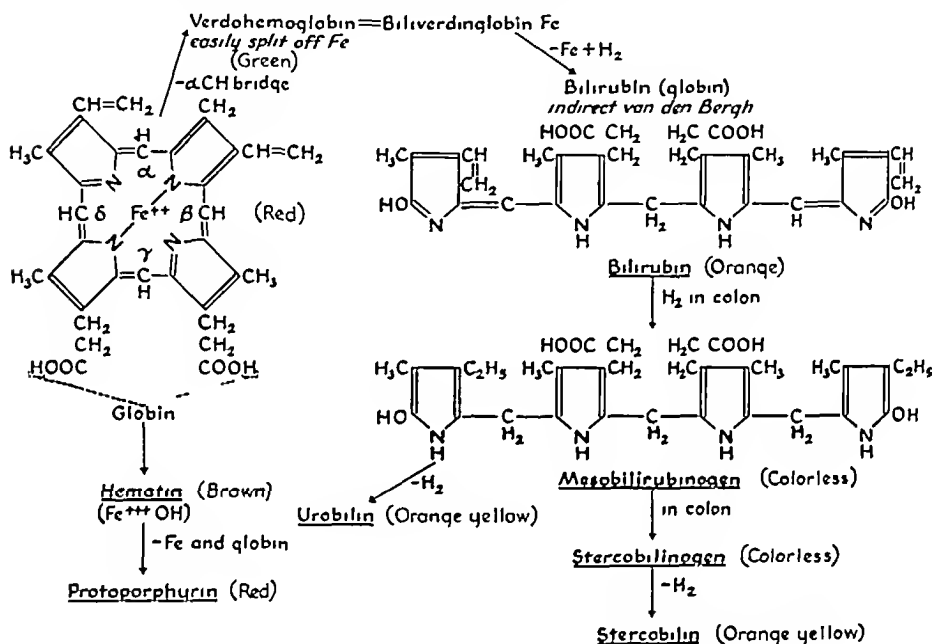


FIG. 1. SOME OF THE MORE IMPORTANT NATURAL DERIVATIVES OF HEMOGLOBIN

sociation. There is no reason to believe, however, that hematin or methemalbumin is a normal intermediary substance in the transition of hemoglobin to bilirubin. Bingold⁹ and Duesberg,¹⁰ in fact, have presented evidence purporting to show that hematin, once formed in vivo, is not converted to bile pigment. An extensive study of this question by Pass, Schwartz, and Watson¹¹ has nevertheless revealed quite clearly that hematin injected into human subjects is converted quantitatively into bile pigment, as judged by a corresponding augmentation of the feces urobilinogen. It appears that free protoporphyrin, in contrast to hematin, is not capable of conversion to bile pigment in vivo, at least in dogs. Watson, Pass, and Schwartz¹² administered protoporphyrin to bile renal fistula dogs, but could observe no significant increase in bilirubin output.

[The studies of H. Fischer,¹³ Warburg,¹⁴ Barkan,¹⁵ Lemberg,¹⁶ and Engel¹⁷ have all indicated that the transition of hemoglobin to bilirubin is over an entirely different pathway than the hematin-protoporphyrin chain as indicated in the lower left corner of figure 1. The studies of Barkan and Lemberg in particular provided evidence that the first step in the transition to bilirubin, rather than the splitting-out of iron or the removal of protein as formerly thought, is the opening of the porphyrin ring by removal of the α -methene bridge. This results in the formation of what is perhaps best termed a verdohemoglobin (Lemberg), or green hemoglobin. As Barkan has shown, the iron in this type of hemoglobin is easily split off in contrast to that of the original hemoglobin molecule in which it is tightly bound.^{15, 18} Barkan and his associates^{14, 19} have provided considerable evidence that up to 5 per cent of the circulating hemoglobin is in this form, at least as judged by the relative amount of easily split-off iron in the circulating red blood cells. Van Havemann²⁰ has described a method for the direct measurement of verdohemoglobin in the red cells, and reports values up to 8 per cent of the total hemoglobin in various individuals. One of the most interesting observations in this connection is that of Barkan¹⁵ that upon the sterile incubation of red blood cells for as little as six hours, a distinct increase of iron and bilirubin in the supernatant plasma is observed without any diminution in the number of cells. The exact mechanism by which the α -methene bridge is removed is not clear. Obviously it is oxidative in character. Engel²¹ has suggested that it is simply a lack of protection by catalase against the activity of H_2O_2 . Van Havemann has shown that verdohemoglobin may be prepared from hemoglobin, *in vitro*, by the action of H_2O_2 .²² According to Siedel,²³ Baumgartel²⁴ has observed the conversion by liver brei *in vitro*. This is reminiscent of the earlier experiments of H. Fischer and Lindner²⁵ in which a green pigment was noted following treatment of hemoglobin by yeast. Siedel,²³ evidently basing his belief upon work by Kiese,²⁶ states that hemoglobin is converted to verdohemoglobin in the liver, *in vivo*.

It appears probable that verdohemoglobin is a biliverdin-iron-globin. It is certain that in the further transition to bilirubin, the iron is split off, and interestingly enough it (the serum iron) travels thenceforth with the globulin fraction of the plasma²⁷ while the bilirubin remains with the serum albumin.²⁷⁻³⁰ The significance of the latter observation, with particular reference to the van den Bergh reaction and bilirubinuria, will be discussed in Part II, which follows.

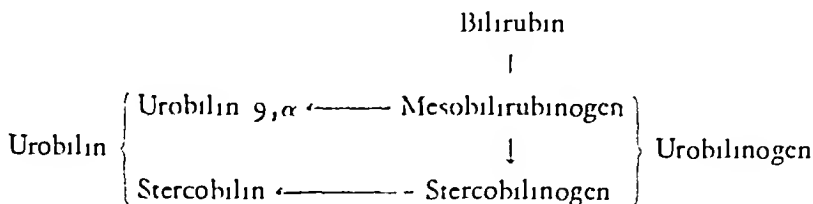
In considering the interesting question of the opening of the porphyrin ring in the transition of hemoglobin to bilirubin, it is evident that if the ring were to open at one or the other of the four methene bridges, a differing bilirubin or bilirubinoid substance would result. It was desirable to identify the bilirubin in human bile of various origin, in order to exclude the occurrence of bilirubins differing from that which had been studied in the past and which, in the main, had been obtained from cattle gallstones.¹ Because of its high melting point, crystalline bilirubin is best identified by one or more of its derivatives. As noted in figure 1, the reduction of bilirubin results in the formation of a colorless chromogen known as mesobilirubinogen, one of the urobilinogens.^{1, 31} The oxidation of mesobilirubinogen results in the formation of urobilin 9, α , an orange-yellow pigment. The designation

9, α , is derived as follows. The classification of the porphyrins, as according to H. Fischer,¹ depends upon the 4 isomers (I-IV) of aetioporphyrin and the 15 (1-15) of mesoporphyrin. These are artificial. The protoporphyrin of the hemoglobin molecule corresponds in configuration to aetioporphyrin III and mesoporphyrin 9. It is a class III type 9 protoporphyrin. The derivatives of protoporphyrin 9, such as bilirubin, mesobilirubinogen, and urobilin all have the same configuration. The second portion of the designation, i.e., α , refers to the opening of the α -methene bridge in the transition of hemoglobin to bilirubin. Urobilin 9, α , is easily crystallized and identified by means of melting point determination.

Watson and Schwartz² subjected a series of human fistula bites to amalgam reduction, thus hydrogenating whatever bilirubin was contained in the samples. The resulting chromogen was extracted and allowed to undergo transition to its corresponding urobilin, by exposure to light. The latter was then crystallized and in each instance was shown to be urobilin 9, α . This indicates beyond doubt that it is always the α -methene bridge which is split out, rather than any of the other three in the protoporphyrin molecule.

The natural reduction of bilirubin to mesobilirubinogen undoubtedly takes place at least mainly in the colon, and is believed to be related to the reducing activity of the bacterial flora. It was long believed that mesobilirubinogen was the only urobilinogen,³ but the isolation of crystalline stercobilin from the feces and urine²¹⁻²³ and its reduction to a chromogen differing from mesobilirubinogen, together with the obvious physical differences between crystalline stercobilin and the urobilin obtained by oxidation of mesobilirubinogen,³¹⁻³⁷⁻⁴² proved beyond question that there are really two urobilinogens in the feces and urine, which, individually at least, are best designated as mesobilirubinogen and stercobilinogen. Collectively they are best referred to as urobilinogen, since both substances are characterized by an intense Ehrlich aldehyde reaction, which has come to be associated clinically with the term urobilinogen. It has now been shown,⁴³ both by feeding of mesobilirubinogen to human subjects and by incubating crystalline mesobilirubinogen with feces, that this substance is converted to stercobilinogen through some as yet unknown agency of the bacterial flora of the intestine. According to Siedel's recent report,²³ Baumgartel²⁴ showed that this conversion is dependent upon the cystin-cystein redox system of the intestinal flora. Present knowledge of mesobilirubinogen and stercobilinogen does not indicate any difference in physiological significance. The relative proportion of the two chromogens varies with different urine samples,⁴⁴ but this as well probably relates to varying bacterial activity in the colon with resultant fluctuation in the amount of stercobilinogen formed. Fortunately, the color intensity of the Ehrlich aldehyde compounds of the two chromogens is identical,⁴⁵ so that the Ehrlich reaction may be used to quantitate them as one. This is another ground, from a clinical standpoint, for use of the single term urobilinogen to indicate the sum of the two chromogens in feces or urine. It may be noted here that Lemberg⁴² employs the terms urobilinogen and urobilin only to designate the substances above referred to as stercobilinogen and stercobilin. Lemberg believed that mesobilirubinogen was not the precursor of these substances, but, as noted above, it has since been shown that it is

converted in the intestinal tract and in the feces, *in vitro*, to stercobilin.⁴³ Lemberg's use of the term urobilin for stercobilin and urobilin β, α , for the urobilin derived from mesobilirubinogen is very confusing especially when it is considered that the stercobilin is just as much a β, α , derivative. It is held, then, that the most suitable classification is as follows:



It is readily seen from the diagram in figure 1 that urobilinogen (as represented by mesobilirubinogen plus stercobilinogen) will be increased in the feces in the presence of an increased rate of hemoglobin catabolism, and decreased under converse circumstances, or when there is an interference with the outflow of bile, so that less bilirubin is permitted to enter the intestine. Thus in the hemolytic anemias, the amount of urobilinogen in the feces is markedly increased, while in hypochromic anemias, which are often associated with a throttling of the rate of blood destruction, the amount is commonly decreased.⁴⁶ In biliary obstruction, especially that due to cancer, little or no urobilinogen may be present in the feces.⁴⁷ Urobilinogen is reabsorbed from the colon into the portal circulation and returned to the liver. In the presence of liver injury or lowered function of the liver cells, varying fractions of the urobilinogen returning in the portal blood are refused and go over into the general circulation to appear in the urine, so that urobilinogenuria may be an evidence of diminished hepatic function. If bilirubin is prevented from entering the intestines because of interference with the outflow of bile, urobilinogen will not be formed and hence will not be found in more than traces in either feces or urine. This is highly characteristic of jaundice due to cancer of the extrahepatic biliary tract. For further details relating to the clinical aspects of urobilinogen excretion, a number of publications which have appeared within the last ten years may be referred to.⁴⁵⁻⁵²

II THE SERUM BILIRUBIN AND BILIRUBINURIA

As noted in part I, it has been shown that bilirubin is attached to the albumin fraction of the serum proteins.²⁷⁻³⁰ This does not say, however, that it has become detached from the original globin of the hemoglobin molecule. It was also noted in part I that the ultra-centrifugal and electrophoretic behavior of globin is entirely similar to that of albumin, so that if the bilirubin were still attached to globin, one would expect to find it with the albumin fraction.²⁹ The amount of globin, of course, would be relatively so small that it would be difficult to detect in the very much larger amount of albumin. It is logical to suppose that bilirubin which is attached to its original globin, as indicated in parentheses in figure 1, is responsible for the delayed or indirect van den Bergh reaction. Duesberg's postulation of a bilirubinglobin¹⁰ is quite in accord with the careful studies of Coolidge³⁰ which indicated

that the indirect reacting bilirubin was attached to the albumin fraction by an actual valence bond while the prompt reacting type is a dissociable complex with some protein in the serum albumin fraction. As indicated in figure 2, bilirubin is divested of its protein on passing from the liver sinusoids to the bile. Whether this is a function of the Kupffer or the polygonal cells of the liver is not known. According to figure 2 the prompt or r' reacting bilirubin returns from the bile capillary to the blood in regurgitation jaundice. Another possibility, which cannot be ex-

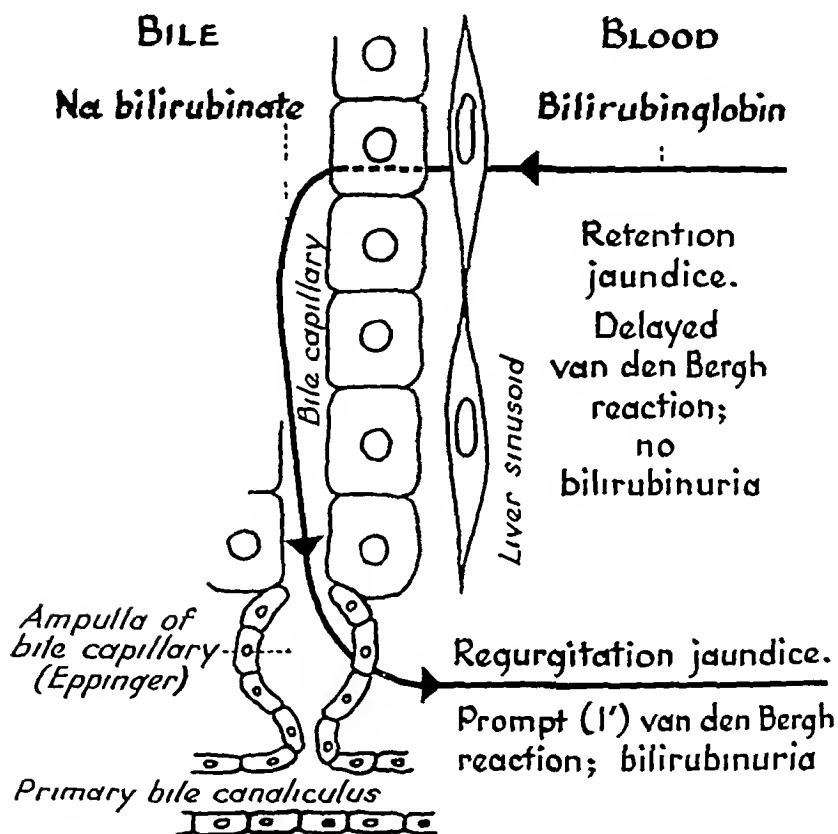


FIG. 2. A CONCEPT OF THE PHYSIOLOGIC BASIS FOR THE PROMPT AND DELAYED VAN DEN BERGH REACTIONS AS SEEN IN RETENTION AND REGURGITATION JAUNDICE, RESPECTIVELY

cluded at present, is that the protein is removed by the Kupffer cells after which the bilirubin is reabsorbed by the polygonal cells and passes into the blood either by way of the lymph or more directly. One may well ask why the bilirubin does not become as tightly bound on returning to the blood from the bile as it was before going through the Kupffer or the liver cells, or both. This cannot be answered with certainty, but it may be due to the fact that bile is weakly alkaline, and it is not unlikely that the bilirubin in the bile is the sodium salt.⁵³ Since the sodium would replace the hydrogen of the two carboxyl groups, it would effectively prevent any

recombination with protein, but it would probably not prevent a loose adsorption. It is necessary to assume that the latter occurs, since the prompt direct reacting bilirubin, as well as the indirect type, is found with the serum albumin fraction^{29,30}. Another point of interest is the chloroform-insolubility of the prompt reacting bilirubin and the relative chloroform-solubility of the indirect or delayed reacting bilirubin³¹⁻³⁵. At first glance it would seem that this ought to be just the opposite, since it has been postulated that the latter is tightly bound to protein. On closer inspection, however, it is readily appreciated that while the sodium salt is chloroform-insoluble, it would nevertheless exhibit a prompt reaction with the van den Bergh (dialzo) reagent, the sodium probably acting to hasten the coupling (see below). Conversely, the chloroform might very well be expected to displace the globin and thus dissolve or extract the delayed or indirect reacting bilirubin. Hunter³² has shown that pure bilirubin suspended in water or alcohol does not react with the dialzo compound but that the addition of as little as 1 per cent of CHCl_3 quickly achieves solution and formation of the azobilirubin. This indicates a striking affinity of chloroform for bilirubin, quite possibly formation of a molecular compound.

The attachment of protein probably affects the type of van den Bergh reaction only in an indirect way, since it is reasonably certain that the diazonium compound does not attach at the carboxyl groups. The exact mode of formation of the azobilirubin is unknown. It has been suggested that a furan ring forms by junction of the vinyl and hydroxyl groups of pyrrol nucleus IV (see fig. 1), thus providing a CH group for reaction with the diazonium salt³⁶. It may be noted, however, that mesobilirubin, which is obtained by reduction of the vinyl groups of bilirubin to ethyl groups, and which, therefore, does not have a structure permitting of furan ring formation, nevertheless couples with the diazonium salt. Furthermore, biliverdin, which still has vinyl groups,^{1,23} does not couple.¹ Bilirubin, mesobilirubin, and mesobilirubinogen, all of which react with the dialzo reagent, differ from the biliverdin in having a central methylene rather than a methene bridge. This in itself suggests that the coupling occurs on the central methylene bridge, and in the case of mesobilirubin it has been shown that the molecule is split in half, each of the two resulting dipyrrolyl methenes having coupled with a molecule of the diazonium compound.¹ Owing perhaps to an increase or decrease, respectively, in the negativity of the charge of the central methylene bridge in the conjugated system which the bilirubin molecule represents,¹ coupling with the diazonium chloride might be expected to be hastened if the bilirubin were a sodium salt, delayed if the carboxyl groups were bound to globin. The effect of alcohol in the indirect reaction might be explained as a loosening of the attachment to protein (whether valence bond or adsorption), thus removing its deterring action on the coupling of the methylene group with the diazonium salt. It is conceivable that the alcohol causes an interchange of sodium with protein, on the carboxyl groups. Another possibility is that the alcohol simply carries the diazonium salt to the methylene bridge much more quickly than it could otherwise get there owing to the physical interposition of the large protein molecules. This would be in better agreement with Coolidge's belief that the alcohol serves as a catalyst, a belief

based on the finding that after addition of alcohol to bilirubin containing serum to make a 50 per cent concentration, as in the Malloy-Evelyn procedure,⁵⁹ the bilirubin was still attached to the serum albumin fraction

Snider and Reinhold⁶⁰ have questioned the existence of any essential difference between the prompt direct and the delayed or indirect reacting types of bilirubin, on the ground that the apparent difference is one of amount only. However, if one plots the reaction curve of an icteric serum with the diazo reagent, the presence of two substances having different reaction times, is clearly indicated (fig 3). If there were but one substance reacting with the diazo compound, a typical parabolic reaction curve would be expected. The contrast between the composite curve of reaction of the prompt and delayed components with the diazo compound, as com-

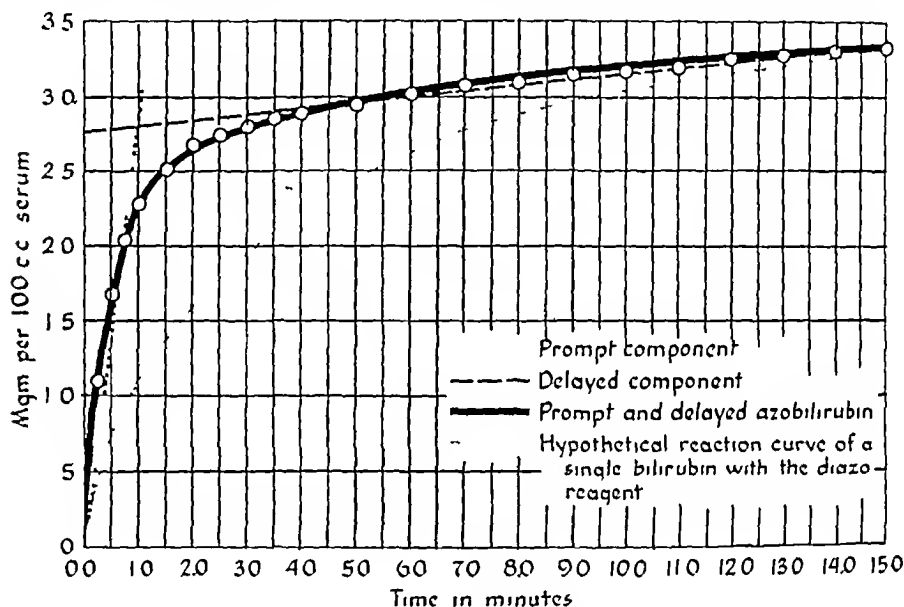


FIG 3 REACTION CURVE OF PROMPT AND DELAYED DIRECT REACTING SERUM BILIRUBIN COMPARED WITH HYPOTHETICAL (PARABOLIC) CURVE OF A SINGLE BILIRUBIN WITH THE DIAZO REAGENT

pared with the hypothetical curve of but two substances is shown in figure 3. It is evident that the change from an almost vertical to a much more horizontal line represents a change from one order of reaction to another, thus indicating the presence of two substances. Similar data have been provided by others.⁶¹⁻⁶³ On the basis of the reaction curve shown in figure 3, it is quite evident that a measurement at one minute is synonymous with the prompt reacting bilirubin, and that for practical purposes one need only measure the one minute and the total,⁵⁷ the latter employing alcohol in accordance with Malloy and Evelyn's procedure.⁵⁹

No information was available in the literature as to the relative fraction of the total serum bilirubin comprised by the one minute or prompt direct reacting type, in normal individuals. Data for 27 normal medical students are given in table 1.

From this it is evident that the upper limit of normal for the 1' bilirubin is unlikely to exceed 0.2 mg. per 100 cc. It is believed that this value should be studied more intensively with relation to the earlier stages of liver injury, particularly of the intrahepatic bile duct system, leading to regurgitation jaundice. Similarly, it is thought that $T - 1'$, or the difference between the 1' or direct and the total bilirubin, probably expresses the retention of bilirubin (globin) by the hepatic cells. This value, in other words, is regarded as a measure of retention jaundice. The data given in table 2 reveal quite clearly the characteristic differences which are en-

TABLE 1 — *The 1' and Total Serum Bilirubins in 27 Normal Medical Students*

No	1 minute	15 minutes	Total (Evelyn Malloy)
1	0.078	0.101	0.768
2	0.094	0.285	0.506
3	0.125	0.252	0.637
4	0.141	0.637	0.900
5	0.047	0.188	1.034
6	0.047	0.252	0.638
7	0.063	0.125	0.474
8	0.110	0.252	0.703
9	0.094	0.252	0.537
10	0.063	0.157	0.443
11	0.094	0.188	0.537
12	0.078	0.157	0.671
13	0.125	0.252	0.571
14	0.063	0.157	0.571
15	0.125	0.252	0.637
16	0.063	0.188	0.506
17	0.125	0.206	0.314
18	0.110	0.379	0.474
19	0.063	0.173	0.251
20	0.000	0.125	0.506
21	0.031	0.188	0.637
22	0.063	0.237	0.637
23	0.047	0.157	0.606
24	0.063	0.141	0.377
25	0.125	0.237	0.443
26	0.063	0.252	0.314
27	0.157	0.303	0.671
Range 0.000-0.157			Range 0.251-1.034

countered in cases of relatively pure retention jaundice as contrasted with regurgitation jaundice of various causes. Slight elevations of the 1' bilirubin are noted in each of the former group. Whether these represent a beginning conversion of the delayed to the prompt reacting type, in other words, a slight overlapping, or whether an actual regurgitation due to mild liver injury, cannot be determined. In any event, the striking difference between the two groups, and the correlation of the 1' bilirubin with bilirubinuria, is obvious.

It is seen from table 2 that in none of the cases of retention jaundice was it pos-

sible to demonstrate any bilirubin in the urine. The sensitive Harrison spot test⁶⁴ was used in all instances. Repeated attempts to detect bilirubinuria were made in case 1, exhibiting a total serum bilirubin content of 14.8 mg, with but 0.59 mg of 1' bilirubin, yet in no instance was a positive test observed. From this it is clear that the renal threshold for bilirubin is not related to the level of the delayed or indirect reacting type. This is in accord with the belief that this type is combined with globin. It appears probable that the renal threshold is related rather to the 1' or prompt reacting type. In this connection it may be noted that while the concept

TABLE 2.—*The 1' and Total Serum Bilirubin in Cases of Retention As Contrasted with Regurgitation Jaundice*

Case no	Diagnosis	Serum bilirubin		Urine bilirubin
		1	Total	
Retention				
1	Infantile cirrhosis	0.59	14.8	0
2	Constitutional hepatic dysfunction	0.96	8.8	0
3	Familial hemolytic jaundice	0.3	3.5	0
4	Familial hemolytic jaundice	0.24	3.2	0
5	Familial hemolytic jaundice	0.71	9.8	0
6	Familial hemolytic jaundice	0.38	5.2	0
Regurgitation				
1	Cirrhosis	1.9	4.0	+
2	Subsiding hepatitis	1.2	2.6	+
3	Common duct stone	6.4	8.4	+
4	Cirrhosis	5.9	11.4	+
5	Carcinoma of pancreas	6.1	8.7	+
6	Carcinoma of pancreas	7.0	44.5	+
7	Carcinoma of pancreas	17.8	31.8	+
8	Acute atrophy	31.9	51.2	+
9	Familial hemolytic jaundice and common duct stone	5.2	11.0	+
10	Common duct stone	20.0	29.4	+

of a correlation between the qualitative direct van den Bergh reaction and bilirubinuria has been appreciated in the past, the statements in the literature relating to renal threshold have quite generally been based upon the total bilirubin, and when reference has been made to the direct reacting type a distinction between prompt and delayed components has been lacking. The data in table 2 raise the question of the renal threshold for the 1' bilirubin. Experience thus far indicates considerable individual variation. This is revealed in the representative data given in table 3.

All of the observations recorded in table 3 were made as the jaundice was diminishing. Neefe and his associates⁶⁵ have observed bilirubinuria in cases of hepatitis at much lower serum bilirubin levels, during the preicteric stage. This suggests that at least in some cases the renal threshold for the 1' bilirubin may be much

TABLE 3 — *Data* Relating to the Renal Threshold of the 1' and Total Bilirubins*

Ca c	1 bilirubin	Total bilirubin	Urine bilirubin
1 B K	1 2	2 6	+
	0 85	1 9	—
2 A W	1 23	2 75	+
	1 00	2 25	—
	0 97	2 00	—
3 S S	0 8	~ 75	—
4 C. B	1 00	3 95	—
	0 7	2 0	—
5 W W	2 0	8 2	+
	1 38	3 0	—
	0 85	2 0	—
6 L. P	2 5	4 7	+
	0 85	2 4	—
7 R B	1 76	4 1	+
8 E P	5 75	12 0	+
	3 0	6 5	—
9 F L.	0 9	2 2	—
10 M B	2 0	4 5	+
	0 6	1 8	—
11 E C.	0 5	1 6	—
12 W M	1 25	3 3	+
	0 4	1 25	—
13 J D	1 3	3 7	+
	0 75	3 4	—
14 R G	2 0	4 8	+
	1 63	3 0	—
15 A E.	1 6	3 1	—
16 B F	0 9	2 2	+
17 L. T	0 8	1 8	+
	0 7	1 4	+
	0 4	1 0	—
18 S O	1 6	3 5	+
	1 4	3 0	—
19 M H	1 6	3 1	—
20 I. T	1 3	2 65	+
	1 4	4 0	—
	1 3	3 9	—
	0 9	2 6	—
21 W S	2 5	4 0	+
	1 5	2 4	—

* Some of these data were obtained during a study of hepatitis carried out at the Schick General Hospital, Clinton, Ia., at the request of the Surgeon General's Office, War Department

lower at the outset than at later stages of the disease. A low renal threshold may well explain the dark urine which is so often noted during the prodromal stage in cases of hepatitis, and which, in the cases without jaundice, may constitute the only sign of regurgitation of bile. It is quite likely that Budd was referring to such

instances when he said, in 1846,⁶⁶ the colouring matter of bile may be detected in the urine, even before the skin becomes yellow, and in some cases the readiness with which it passes off in the urine, seems to prevent the occurrence of jaundice—the skin retaining its natural color while the tint of the urine attests the presence of bile. The data in table 3 reveal again, quite clearly, that the total bilirubin is commonly above 2.0 mg per 100 cc in cases of jaundice due to liver disease, without demonstrable bilirubinuria. The lowest level of the 1' bilirubin in this group, at which bilirubin was present in the urine, was 0.7 mg (case 17). Interestingly enough this was the earliest instance of jaundice in the group, a case of hepatitis studied soon after onset. Data have been made available* from another case of hepatitis studied on the first day of jaundice, in which similar results were obtained. This was in a male of 28 who complained of anorexia, nausea and vomiting, fever and discomfort in the right upper abdomen, all of several days' duration. On the day that he was first studied a slight icterus was believed to be present and bilirubin was detected in the urine, although the 1' bilirubin was 0.6 and the total bilirubin 0.9. On the next day the 1' bilirubin had risen to 1.2 and the total to 2.3.

It is also of interest to observe in the data shown in table 3 that during the late defervescent stage of the jaundice due to hepatitis, bilirubin may disappear from the urine at surprisingly high levels, even of the 1' bilirubin. The outstanding example of this apparent alteration in renal threshold is noted in case 8, a negative urine bilirubin with a 1' bilirubin of 3.0 mg per 100 cc. In the main, however, the threshold in the defervescent stage is between 0.8 and 1.3 mg.

III THE ERYTHROCYTE PROTOPORPHYRIN

The presence of free protoporphyrin in the erythrocytes was first reported by van den Bergh and Hyman in 1928.⁶⁷ This observation was soon confirmed.⁶⁸⁻⁷⁰ Subsequent studies have indicated that one or more of at least three factors appear to be concerned in determining the amount of protoporphyrin which will be found in the erythrocytes of a given individual. These are as follows: (1) Increased reticulocyte percentage, or normoblastic hyperplasia in the bone marrow, the term normoblast indicating only the later stage of erythropoiesis in which hemoglobin manufacture is under way. (2) The presence and degree of iron deficiency or of factors interfering with the utilization of iron in the synthesis of hemoglobin, as for example, lead. (3) The formation of protoporphyrin from hemoglobin in the erythrocytes.

It has been amply demonstrated that the reticulocytes are rich in protoporphyrin^{71, 72} but that the protoporphyrin concentration of the red cells is not always correlated with the reticulocyte percentage.⁷³ In induced hemolytic anemia, as for example in acute phenylhydrazine anemia of the rabbit, the reticulocyte curves closely follow those of protoporphyrin concentration. On the other hand, in cases of pernicious anemia followed before, during, and after liver therapy, it was shown⁷³ that the peak protoporphyrin concentrations were usually reached sometime after the peak reticulocyte response (see fig. 4). This finding will be considered in more detail below. It is in accord with the previous observations of

* Through the courtesy of Capt. Emanuel Rappaport, M.C. A.U.S. Chief of the Gastrointestinal Service, Schick General Hospital, Clinton, Iowa.

Seggel and co-workers^{74 75} on the percentage of fluorescytes as affected by liver therapy in pernicious anemia. According to this and the studies of Chytrek⁷⁶ and others, the fluorescytes are red blood cells exhibiting a red fluorescence in ultra-violet light, presumably due to their content of protoporphyrin.

The normal range of concentration of the EP (erythrocyte protoporphyrin) is from 15 to 40 γ per 100 cc of erythrocytes,⁷³ usually below 30 γ . Values up to 40 γ are noted in apparently healthy females but are correlated with mild reductions in hemoglobin and hematocrit percentage probably on the basis of blood loss during menstruation.⁷³ Iron deficiency anemia is characterized by marked increases often

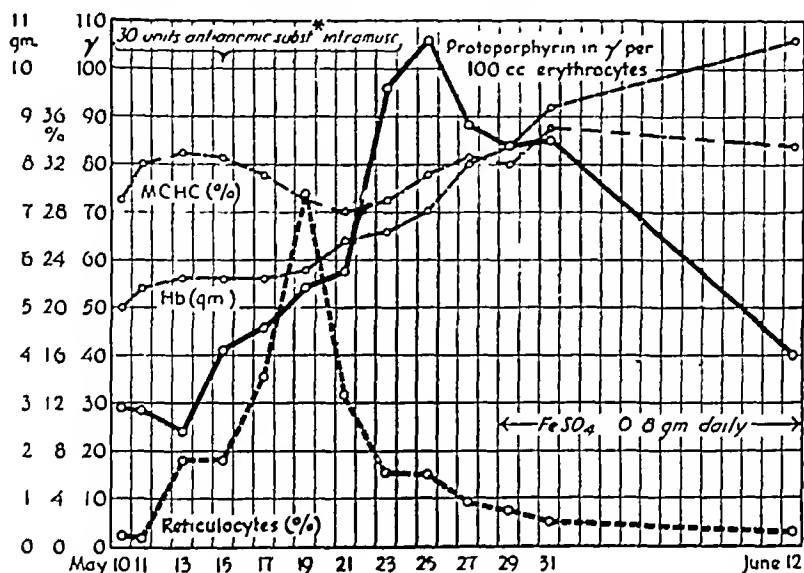


FIG 4 ERYTHROCYTE PROTOPORPHYRIN CONCENTRATION, RETICULOCYTE PERCENTAGE, HEMOGLOBIN IN GRAMS PER 100 CC. OF BLOOD, AND HEMOGLOBIN CONCENTRATION OF THE ERYTHROCYTES BEFORE AND AFTER LIVER EXTRACT THERAPY IN A CASE OF PERNICIOUS ANEMIA (From Watson, Grinstein, and Hawkison Studies of protoporphyrin IV J Clin Investigation 23 69, 1944) C S, M, 53

* Liver concentrate, 10 units per cc, intramuscularly, on May 10, this dose was repeated on May 27

of 10 to 20 fold. In the main, a rough inverse relationship exists in these cases between the hemoglobin concentration of the red cells and the amount of protoporphyrin (table 4). It is seen, however, that in certain instances (cases 51 and 52) the values are not as elevated, in spite of a more severe anemia and greater reduction of hemoglobin concentration, as they are in others (cases 37 and 44). The impression has been gained that a better correlation exists with the degree of chronicity as indicated by the history, and by the presence of the epithelial changes associated with iron deficiency, i.e., dry hair and skin, changes in the finger nails, glossitis, dysphagia. Cases 37, 44, and 47 were illustrative.

The EP in pernicious anemia in relapse is uniformly within the normal range. This is shown by the data in table 5. These values are in striking contrast to the

TABLE 4—*Erythrocyte Protoporphyrin in Iron Deficiency Anemia*

Case no	Hemoglobin in Gm per 100 cc	Hematocrit (%)	M C C	Reticulocyte (%)	Protoporphyrin in γ per 100 cc of erythrocytes
34	9 8	36	30	0 9	221
35	9 45	29 4	32	1 -	103
36	7 45	24	31	0 7	50
37	5 75	23	25	6 2	613
38	7 00	28	25	- 2	143
39		30		0 6	117
40	9 75	35 5	27 4	0 9	165
41	5 25	18	29	1 2	77
42	9 45	32 5	29	4 0	70
43	8 3	28 5	29	2 8	137
44	10 95	40	27	2 0	320
45	9 5	33	29	1 8	208
46	7 3	31 8	23	2 1	142
47	6 18	29	21 4	5 9	504
48	8 95	31	29	0 9	309
49	6 87	24 6	28	1 5	15
50	7 8	34	23	1 8	221
51	4 13	19	21 7	1 9	253
52	4 55	18	25 3	5 1	172
53	8 3	31 5	26	8 3	128

TABLE 5—*Erythrocyte Protoporphyrin in Pernicious Anemia (Relapse)*

Case no	Hemoglobin in Gm per 100 cc	Hematocrit (%)	M C C	Reticulocyte (%)	Protoporphyrin in γ per 100 cc of erythrocytes
1	5 06	18 7	27 1	1 0	29
2	9 0	23	39 1	0 9	23
3	5 0	17	32	1 0	29
4	5 0	19	26 3	0 3	22
5		37		1 1	15 7
6	3 44	11	31 2	0 3	16
7	7 8	25	31 2	0 5	23 5
8	10 45	38	27 5	0 6	20
9	4 63	15	31	1 1	20
10	6 8	21 5	31 6	0 7	27
11	8 07	27	30	1 8	20
12	3 44	11	31 2	0 1	30 3
13	6 95	23	30 2	1 0	20
14	8 8	22	40	1 0	27
15	4 67	15	31	1 1	35

findings in the vast majority of anemias of other types, notably the iron deficiency anemias and the refractory or hyporegenerative variety. Of the seven cases of refractory anemia listed in table 6, it is seen that five had considerably elevated EP values. It is apparent that iron deficiency was not the cause of these elevations,

since the mean corpuscular hemoglobin concentrations were either approximately normal or within the normal range. The cause of the elevation in these cases is not at all clear. It is evident that the increase cannot be ascribed to an increase in reticulocytes. In case 59, for example, the reticulocyte count was 0.8 per cent with an EP of 74 γ . In this instance, the mean corpuscular concentration of hemoglobin

TABLE 6—*Erythrocyte Protoporphyrin in Refractory (Hyporegenerative or Aplastic) Anemia*

Case no	Hemoglobin in Gm per 100 cc	Hematocrit (%)	M C C	Reticulocyte (%)	Protoporphyrin in γ per 100 cc of erythrocytes
54	6.25	19.6	32	2.3	76
55	7.1	23	31	0.9	147
56	7.98	27.5	29	1.0	36
57	5.17	17	30.2	2.1	94
58		8			20
59	4.17	12	35	0.8	74
60	8.3	24	34.6	3.2	132

TABLE 7—*Erythrocyte Protoporphyrin in Leukemia, Hodgkin's Disease, Polycythemia, Myeloma, Lymphosarcoma*

Case no	Diagnosis	Hemoglobin in Gm per 100 cc	Hematocrit (%)	M C C	Reticulocyte (%)	Protoporphyrin in γ per 100 cc of erythrocytes
61	Myelogenous leukemia	8.15	30	27.2	3	78
62	Myelogenous leukemia	8.65	30.8	28.1	0.3	56
63	Myelogenous leukemia	9.02	34	26.5		40
64	Myelogenous leukemia	10.65	31	34.4		48
65	Lymphatic leukemia	12	37	32.5		23
66	Lymphatic leukemia \bar{c} Acquired hemolytic anemia	6.15	24	25.6	2.8	119
67	Lymphatic leukemia	3.23	10	32.3	3.5	40
68	Multiple myeloma	7.52	24.5	31	3.4	126
69	Multiple myeloma	11.5	38	30.3		31
70	Lymphosarcoma	6.94	24	28.6	1.5	171
71	Hodgkin's disease	8.6	32	27	4.0	44
72	Hodgkin's disease	10.95	35.5	31	1.0	70
73	Polycythemia vera	17.5	64	27	1.5	68
74	Polycythemia vera	19.6	65	30	0.8	40
75	Polycythemia vera		68		0.5	24
76	Polycythemia vera		61.5		0.6	39
77	Polycythemia vera	13.5	41.5	32.5	0.8	46

was 35 per cent so that there was no evidence of any iron deficiency. In such instances as this, it is possible that the third of the three factors mentioned at the outset may be operative, viz., the formation of protoporphyrin from hemoglobin within the erythrocytes. Other evidence in favor of this possibility is the observed increase of protoporphyrin in the erythrocytes upon sterile incubation of blood *in vitro*.⁷³ It is possible that this might occur wherever red blood cells are sequestered,

as, for example, in the splenic pulp. Further study of this question is needed. In a previous report,⁷ a case of myeloid leukemia with severe myelophthisic anemia was recorded in which no reticulocytes could be demonstrated in the peripheral blood on repeated occasions. The color index was 1.0. Here it was evident that neither iron deficiency nor reticulocytes were contributing to the presence of the protoporphyrin in the red blood cells, the amount of which was 43 γ per 100 cc. It may be inferred that formation from hemoglobin had occurred, although proof of this derivation is lacking.

TABLE 8—*Erythrocyte Protoporphyrin in Heavy Metal Poisoning*

Case no	Diagnosis	Hemoglobin in Gm per 100 cc	Hematocrit (%)	M C C	Reticulocyte (%)	Protoporphyrin in γ per 100 cc of erythrocytes
79	Pb poisoning	10.0	34	29.4	14.6	240
80	Pb poisoning	6.6	18.5	35.7	1.9	470
81	Arthritis patient receiving gold therapy	9.45	27	35	0.7	107

TABLE 9—*Erythrocyte Protoporphyrin in Hemolytic Jaundice*

Case no	Hemoglobin in Gm per 100 cc	Hematocrit (%)	M C C	Reticulocyte (%)	Protoporphyrin in γ per 100 cc of erythrocytes
16	12.1	37	3~7	7.1	48
17	5.35	21.5	25	49.7	144
18	9	33	27.2	7.1	60
19	6.75	24	29	31	86
20	11.7	32	33	5.9	37
21	2.73	8	34.2	5.3	42
22	8.15	31.5	26	8.2	122

An EP value of 30 γ per 100 cc of erythrocytes has recently been observed in a case of advanced hepatic cirrhosis in which there was also a severe anemia of macrocytic, but nonhemolytic type (feces urobilinogen 43 mg per day, reticulocytes 0.8 per cent). The sternal bone marrow in this case was scanty and difficult to obtain, normoblasts were relatively scarce, and no megaloblasts were seen. No benefit was observed from liver extract therapy. This is one of the few instances of anemia thus far observed in which the EP value was within the normal range, although pernicious anemia was excluded.

Of the group of cases in table 7, case 68 is of particular interest, since this patient was thought at first to have pernicious anemia, the peripheral blood exhibiting macrocytosis and an almost normochromic state. The high EP pointed to another diagnosis, and the bone marrow aspiration subsequently revealed the typical appearance of multiple myeloma rather than pernicious anemia. It is of interest to note that of the five cases of polycythemia vera, the only distinctly abnormal value, that of 68 γ per 100 cc in case 73, was associated with a significant lowering of the

mean corpuscular hemoglobin concentration to 27 per cent This patient had been bled repeatedly and undoubtedly suffered from iron deficiency

In table 8, data from three cases are given in which elevations of the protoporphyrin in the red cells were associated with heavy metal poisoning Case 80 was of particular interest because of the coexistence of peripheral neuritis Occasional stippled cells were found in the peripheral blood after prolonged search They were more numerous in the bone marrow, which was normoblastic in type The markedly increased EP in the absence of iron deficiency suggested lead poisoning, and upon further questioning it was ascertained that this individual had had considerable contact with tetra-ethyl lead and with batteries while working as a filling station attendant Subsequent study of the urine revealed in the neighborhood of 1000 γ of coproporphyrin per day, at least ten times the normal range This increase was shown to be composed of the type 3 isomer as noted previously in cases of lead poisoning^{77 78}

The EP is usually distinctly increased in the hemolytic anemias, as contrasted with pernicious anemia (see table 9) An unusual case of spheroidocytic hemolytic anemia has been studied recently (not included in table 9), in which the reticulocyte percentage was consistently very low, in the neighborhood of 0.2 per cent The EP was 72 γ per 100 cc at a time when the hemoglobin was 4.2 Gm, the erythrocytes 1,000,000 per cu mm After transfusions the spleen was removed On the eighth postoperative day the EP was 20 γ per 100 cc

The lowest EP value that has been noted in the hemolytic anemia group before splenectomy is that of case 20 in table 9, i.e., 37 γ per 100 cc This is in striking contrast with the regularly lower values observed in pernicious anemia (table 5) In general, the following ranges of concentration are compatible with one of the anemias in the group indicated

	Erythrocyte protoporphyrin concentration
Pernicious anemia	15-30 γ %
Hemolytic anemia	30-150 γ % Usually 50-100
Refractory or hyporegenerative anemia	
Leukemia, Hodgkin's disease, myeloma	
Iron deficiency anemia	50-600 γ % Usually greater than 100 γ %
Anemia due to heavy metal toxicity	

DISCUSSION

The experimental and clinical evidence already referred to which relates an increased EP to an increased percentage of reticulocytes is in accord with Stasney's direct observations of the protoporphyrin content of the bone marrow⁷⁹ These have indicated that normoblasts contain protoporphyrin in considerable amount, while megaloblasts (as in pernicious anemia) do not It was previously suggested⁷² that Stasney's observations might explain the delayed rise of the EP as compared with the reticulocyte peak following liver extract therapy (see fig. 4) Regardless

of whether the pernicious anemia megaloblast is regarded as a precursor of the normoblast or as a peculiar and independent cell line which rapidly disappears after administration of liver extract, the delayed rise of the EP is entirely compatible with Stasney's findings. One of the chief characteristics of pernicious anemia megaloblasts is the relatively rapid rate at which hemoglobin has developed. Cells with apparently normal hemoglobin concentration often exhibit relatively *immature nuclei*. This might well be correlated with a much more complete utilization of protoporphyrin as rapidly as the latter is synthesized, so that at no time is any appreciable amount left free either in the marrow or in the resulting megakaryocytes which gain access to the peripheral blood. All of this may be viewed simply as a part of the characteristic 'maturation arrest' of pernicious anemia bone marrow. The EP in a typical case of Cooley's erythroblastic anemia (female, aged 7) was recently determined and found to be 20% per 100 cc. This finding is of particular interest since, if confirmed, it would indicate that protoporphyrin is not formed in appreciable amounts at the erythroblast stage. The combined available evidence indicates that the main formation of protoporphyrin is closely associated, as might be expected, with hemoglobin formation in the maturing normoblasts. It remains to be determined whether the protoporphyrin of the reticulocytes is merely a small excess which was left over, as it were, during the synthesis of hemoglobin, also what the ultimate fate of this free protoporphyrin is, whether eliminated or built up into additional hemoglobin in the circulating erythrocyte.

Because of a co-precipitation of brilliant cresyl blue and protoporphyrin, *in vitro*, at pH 7.3, it was suggested in an earlier paper⁷¹ that protoporphyrin might be fundamentally related to the supravital staining of the reticulocytes. The subsequent finding that an increased EP might be observed without a high reticulocyte percentage appeared to make this possibility less probable. In no instance, however, *has an elevated reticulocyte count been noted without a distinct increase of the EP*. If one considers the factors mentioned at the outset—i.e., (1) increased reticulocyte percentage or normoblastic hyperplasia, (2) iron deficiency or interference in iron utilization, and (3) liberation of protoporphyrin from hemoglobin in intact erythrocytes, as in the splenic pulp—then it is evident that, depending on which factor was of chief importance in a given case, the protoporphyrin might be expected to be free or bound to protein. If the increased EP were related simply to increased normoblastic activity in which, owing to acceleration of erythropoiesis, small fractions were not being put together into hemoglobin, one might well expect that the protoporphyrin would be free. With the remaining factors mentioned, protoporphyrin would probably be bound to protein but not to iron, in which event it might be expected to elevate the value in the chemical determination but have no effect in the supravital staining process. While this is admittedly speculative, it indicates the need of further investigation, as well as the general direction which it should take.

SUMMARY AND CONCLUSIONS

The transition of hemoglobin to bile pigment, at least under normal conditions, is believed to occur via an intermediate biliverdin-globin-iron (verdohemoglobin)

and not over the stages of hematin and protoporphyrin. It is probable that the next step is a reduction to bilirubin with splitting off of iron. There is much reason to believe that the globin remains attached until the bilirubin passes through the liver cell, *bilirubinglobin* exhibiting a delayed or indirect van den Bergh reaction and not being excreted in the urine, the *sodium bilirubinate* of the bile exhibiting a prompt (r') van den Bergh reaction and being readily excreted in the urine. The former type is characteristic of retention, the latter of regurgitation jaundice.

The appearance of bilirubin in the urine is believed to be related to the concentration in the blood of the r' or prompt bilirubin, rather than that of the total bilirubin. It is evident that the threshold may be considerably lower at the onset of jaundice, as, for example, in hepatitis, than during its defervescence. This undoubtedly accounts for the appearance of bilirubinuria prior to recognizable jaundice in certain instances, likewise for its presence in the cases of so-called hepatitis without jaundice. In retention jaundice marked elevation of the total serum bilirubin is unassociated with bilirubinuria, in these cases the increase of bilirubin is mainly of the delayed or indirect reacting type. Further evidence is presented of the essential difference between the r' or prompt, and the T minus r' , or delayed and indirect reacting bilirubins. This consists of a change of the order of reaction at one minute after adding the diazonium salt. The normal upper limit of the r' bilirubin has been shown to be in the neighborhood of 0.2 mg per 100 cc, figures well below this value are usually obtained.

Further experience with the erythrocyte protoporphyrin in the anemias has revealed that this determination, quite apart from its fundamental interest, is at times of diagnostic value. Thus in several instances a significant elevation of the erythrocyte protoporphyrin has indicated that the initial impression of pernicious anemia was incorrect, and has led to the search for other information. Conversely, a low normal value in the presence of anemia has often correctly indicated or confirmed the diagnosis of pernicious anemia. Marked elevations have aided in confirming the presence of iron deficiency and have given some insight into the degree of its severity and chronicity. In certain cases, high values for the erythrocyte protoporphyrin have suggested the possibility of heavy metal toxicity, the existence of which has then been borne out by subsequent study.

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THE CARDIOVASCULAR SYSTEM IN ANEMIA

WITH A NOTE ON THE PARTICULAR ABNORMALITIES IN SICKLE CELL ANEMIA

By MAXWELL M. WINTROBE, M D

THAT the cardiovascular system is influenced by anemia is generally recognized. Almost sixty years ago Strumpell¹ noted palpitation as one of the first symptoms of severe anemia. He observed that breathing is hurried, owing to a feeling of air hunger and shortness of breath, and that a feeling of oppression in the breast is characteristic. Bamberger² nearly a century ago pointed out that cardiac enlargement is found in many patients suffering from anemia. The extent to which the functional activity of the cardiovascular apparatus is affected by anemia, however, is not so generally appreciated. Changes in the heart and circulation develop in part in response to needs arising from the lack of hemoglobin and thus of oxygen per unit of blood, and in part as a result of the influence of the anemia on the heart itself.

Rapid development of anemia is accompanied by shortness of breath, tachycardia, and pallor. Very severe anemia, if produced quickly, as after severe blood loss, results in shock. Here we deal with a failure of adjustments to the lack of oxygen-transporting material as well as with the effects produced by a sharp reduction in total blood volume. In cases in which serious cardiac damage is already present, coronary thrombosis or cardiac failure has been known to develop after severe hemorrhage.³ In chronic anemia, on the other hand, a severe grade of anemia seems to be well tolerated in many instances. This is no doubt due to the maintenance of an essentially constant blood volume as well as to the opportunity for physiologic adjustments. It is to cardiovascular manifestations associated with chronic anemias that chief reference will be made here.

In chronic anemia the symptoms may be merely moderate dyspnea and palpitation, or there may be tachycardia and precordial pain. Physical findings may include edema of the ankles, apical systolic murmur, lateral displacement of the left cardiac border, downward extension of the liver border, basal systolic murmur, basal rales, and liver tenderness. In a series of 300 cases of pernicious anemia, Carter and Traut⁴ found these symptoms and signs appearing in frequency in the order named.

Several writers⁵ have reported cases in which the initial and presenting complaints in a case of chronic anemia were those of *congestive cardiac failure*, and every internist of some experience can recall such cases. In the Indian Medical Service, Gunewardene⁶ called attention to the frequency of heart failure in ancylostomiasis. In these cases treatment and cure of the infection and the associated anemia were

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accompanied by disappearance of symptoms of failure, reduction in size of the heart, and disappearance of the murmurs

Herrick (1918)⁷ was one of the first to call attention to the association of pernicious anemia and *angina pectoris*. Cary Coombs⁸ in 1926 found this combination in 9 of a series of 36 cases of pernicious anemia. In Cabot's cases⁹ it was demonstrated that the symptoms of angina occurred in persons showing no changes in the coronary arteries. Pickering and Wayne¹⁰ described cases of *intermittent claudication* as well as *angina pectoris* in association with anemia, and Zimmermann¹¹ has also reported *angina pectoris* in association with severe anemia. There is no doubt that in many of the reported cases, the background for the development of cardiac dysfunction existed before severe anemia developed. Nevertheless, the symptomatic improvement that followed relief of the anemia, speaks for the significance of the anemia in precipitating the cardiac symptoms. Thus in 6 of Pickering and Wayne's 8 cases, the anginal pain did not continue after the anemia was cured, and the tolerance to exercise improved as the hemoglobin level rose. Claudication disappeared permanently in 6 of 7 cases after relief of the anemia. Significant also in regard to the role of anemia as a cause of angina is the fact that, among cases of angina associated with anemia, females have presented this symptom as often as or more often than males, this is in marked contrast to the sex incidence of angina associated with organic heart disease. Furthermore, the mortality of anemic patients with angina is low—approximately from 3 to 10 per cent is compared with about 40 per cent for cases of anginal pain associated with organic heart disease.

Cardiac enlargement in anemia, without other etiologic basis, has been observed repeatedly. The largest heart weighed 710 Gm.⁹ Porter¹² observed a heart weighing 630 Gm. in a man who died of hookworm anemia. The heart is often symmetrically enlarged. German and Austrian clinicians of the nineteenth century studied this subject and noted that the heart became smaller when the anemia was relieved. The return to normal size may occur within a very few weeks.¹³ Cardiac enlargement has been observed more frequently in patients with particularly low hemoglobin levels and also in children, as well as in the oldest age groups. This has also been demonstrated in association with anemia produced by hemolysis and in anemia produced experimentally, as well as in miscellaneous forms of anemia. The greater the degree of the anemia, the more likely is the heart to decrease in size after improvement. Dilatation as well as hypertrophy must be a factor in the cardiac enlargement. It seems likely that anemia of short duration results in cardiac dilatation that can be completely overcome with relief of the anemia, whereas in cases of long duration hypertrophy takes place.

That *systolic murmurs* frequently develop in patients suffering from anemia is well known. In one series⁴ of cases, apical systolic murmurs were the most frequent, in another,¹³ the murmurs were equally divided between the apex of the heart and the area about the second or third interspace to the left of the sternum. Next in frequency are aortic systolic murmurs, these are high-pitched and blowing in character. In children¹⁴ the apical systolic murmur may be transmitted to the axilla and even to the back. Diastolic murmurs, though much less frequent, may occur in the absence of organic heart disease. The usual diastolic murmur is early and blowing

in character and is best made out in the third left interspace near the sternal border. It may be basal or apical in location. Diastolic murmurs have been described only in association with very severe grades of anemia.¹³

In many cases of severe anemia, *electrocardiographic changes* have been noted.^{4, 13} The most common change is depression of the R-T (S-T) junction, with a u shaped deformation of the S-T segment and flat or inverted T waves, but without corresponding changes in the QRS complex. The changes are in no way specific and resemble those known to occur in severe cardiac anoxia (coronary insufficiency) or those seen when toxic myocardial effects are present. They often resemble the changes produced by digitalis (changes in the ventricular gradient of the heart muscle). Changes in the deviation of the electrical systole (Q-T interval) and disturbances in AV conduction have been noted occasionally. Such alterations in electrical conduction have been observed chiefly when the anemia was very severe, with a hemoglobin level of 4 or 5 Gm. or less. Reversal of these changes when the blood was restored to normal has occurred.

In any given case of anemia the manifestations related to the cardiovascular system will naturally depend on many factors, namely (1) the degree of anemia, (2) the rapidity of development of anemia, (3) the age of the patient and the capacity of the cardiovascular system for adjustment, (4) the previous state of the cardiovascular system.

THE PHYSIOLOGIC ADJUSTMENTS TO ANEMIA

In the accompanying diagram (fig. 1) an attempt is made to indicate the physiologic adjustments to anemia that take place in the cardiovascular system. This is purely schematic, for the exact details and quantitative relationships have yet to be worked out. Important contributions on this subject have been made by Blumgart and his associates,¹⁵ by Stewart and his co-workers,¹⁶ and by a few other investigators.^{17, 18, 19} The most recent study of note is that of Sharpey-Schafer.²⁰ The clinical evidences of an adjusting circulation in cases of anemia are to be found in the rapid heart rate, increased arterial pulsation, increased pulse pressure, and even capillary pulsation in the finger tips. Sharpey-Schafer,²⁰ noting this hyperkinetic syndrome, as Harrison calls it, described pistol shot sounds over the arteries in his cases, positive Duroziez sign, and systolic murmurs on auscultation of the eyeball.

An increase of cardiac rate and in the velocity of blood flow results in greater minute-volume output. The circulation time is reduced. It has been shown repeatedly that the cardiac output is increased in anemia. This may be preceded by a rise in pressure in the right auricle.²⁰ At what level of anemia a significant increase occurs is not so certain. Some investigators set this level at 50 per cent hemoglobin. The increase in cardiac output when the hemoglobin has fallen to 30 per cent has been said to be as much as 200 per cent. Employing the cardiac catheterization technique and the direct Fick method, Sharpey-Schafer²⁰ observed cardiac outputs varying from 7.4 to 13.4 liters per minute in cases of posthemorrhagic anemia with hemoglobin levels that varied from 25 to 68 per cent, as compared with a normal average cardiac output of 5.3 liters per minute.

The falling viscosity of the blood, lowered arterial blood pressure, and decreased peripheral resistance tend to reduce the work of the heart. According to the studies of Stewart, Crane, and Deitrick¹⁶ in 5 cases of pernicious anemia, various opposing factors operate so that the work of the heart is not increased in spite of increased cardiac output. The total blood volume may be slightly reduced in anemia,¹⁸ although it is not lowered as much as is suggested by the fall in red cell volume, because plasma volume increases to some extent. Whether or not the work of the heart is increased in any case probably depends on additional factors and may differ from case to case. The fact that the heart has been found to be enlarged and hyper-

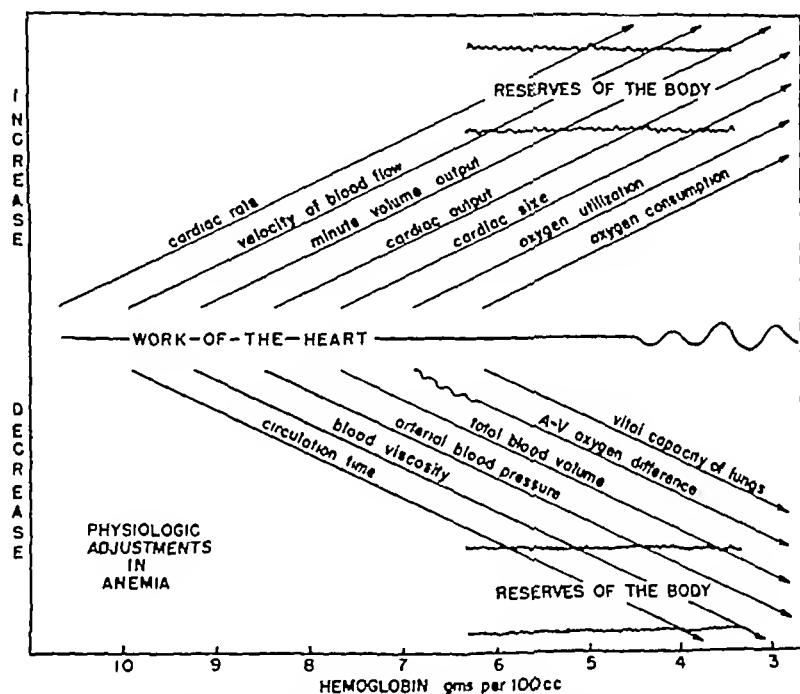


FIG. 1. SCHEMATIC DIAGRAM OF PHYSIOLOGIC ADJUSTMENTS TO ANEMIA

At first these are so balanced that work of heart is not changed. With increased demands for adjustment, reserves of body are encroached upon and cardiac failure may ensue.

trophied in a number of cases of anemia, has led to the assumption that the work of the heart has been increased. Cardiac hypertrophy in anemia has also been attributed to insufficient oxygen supply to the myocardium.

Increased oxygen utilization represents another means of physiologic adjustment to anemia. By this is meant the removal by the tissues of a greater proportion of the oxygen carried to them. Of the 18-21 volumes per cent of oxygen carried by the arterial blood, no more than 5-5 volumes per cent are ordinarily given up in the capillaries to the tissues. Were it not for increased oxygen utilization, cardiac output would have to reach even higher figures to maintain an adequate oxygen supply. The arteriovenous oxygen difference has been observed²⁰ to fall in anemia to

between 19 and 41 cc per liter, as compared with a normal average of 45 cc per liter

When the hemoglobin level of the blood is low, say about a third of normal, only 6 volumes per cent of oxygen are being carried by the blood. When anemia of this degree has developed, even if oxygen utilization is very greatly increased, the amount of oxygen available is seriously reduced. Furthermore, much of the oxygen load of the blood in such a case would be supplied to the tissues at a very low pressure—6 volumes per cent minus, for example, approximately 5 volumes per cent—which would result in anoxia, since this pressure is too low to be efficient. An increased circulation rate is beneficial here, because each unit of blood probably gives up a smaller proportion of its oxygen load, and thus oxygen is delivered to the tissues at a higher pressure than would otherwise be possible. Thus the arterio-venous oxygen difference, although it may at first be greater because of increased oxygen utilization, subsequently is reduced because of changes in circulation rate.

In anemia the vessels of the skin may be constricted, thus forcing a greater proportion of the blood through other regions. The oxygen consumption (basal metabolic rate) may be increased. Several investigators have noted a decreased vital capacity of the lungs. The reason for this is not evident, unless it is due to the presence of an increased amount of blood in the lungs coincident with an increase in the rate of blood flow.

The physiologic adjustment in anemia may be so efficient that a patient with only 15 Gm of hemoglobin and 8 cc volume of packed red cells per 100 cc of blood, can manage to get out of bed and walk slowly about the room. I have seen a nurse permit such a patient to go by herself to the bathroom, and it was I who was shocked, rather than the patient, when I discovered how anemic the latter was.

It must be evident, however, that the physiologic adjustments resorted to by the anemic individual call for considerable encroachment on the reserves of the body. It is not surprising that such a patient may become short of breath on exertion or that symptoms of cardiac failure or of angina may appear. The edema found in association with anemia may be due to this cause, or to hypoproteinemia, or it may be independent of these. Venous pressure is not usually increased in cases of anemia unless congestive cardiac failure is present. According to the studies of Strauss and Fox,¹⁹ anemia per se, in some unexplained way, may be a factor conducive to water retention.

Much further study is required to determine whether the physiologic adjustments that have been described take place in all types of anemia. * Most of the studies heretofore have been made in cases of pernicious anemia. Blumgart and his associates¹⁵ found that the velocity of the pulmonary blood flow was relatively slower in patients with anemia associated with carcinoma than in pernicious anemia at corresponding levels of hemoglobin. For this reason, they concluded, dyspnea, signs of

* The excellent studies by Brannon, Merrill Warren, and Stead (*J. Clin. Investigation* 30: 332, 1945) and by Sharpey Schaefer (*Lancet* 2: 296, 1945) published since this paper was written, show clearly that increased cardiac output, increased oxygen utilization, and decreased peripheral resistance are present in cases of severe anemia.

congestive failure, peripheral edema, weakness, and cyanosis are frequently more pronounced in patients with carcinoma than would be expected on the basis of anemia, malnutrition, or toxicity. This is a problem for further investigation.

SICKLE CELL ANEMIA

There is one type of anemia that deserves special mention when the relation of the heart to anemia is being considered. This is sickle cell anemia. This strange disorder, seen—with certain rare exceptions—only in the Negro race, is characterized by the peculiar shape assumed by the red corpuscles when they are deprived of oxygen. The symptoms of this anemia are like those of any chronic anemia, but in addition the patients may suffer from attacks of severe pain in various parts of the body, especially in the extremities and about the joints. This complaint may appear following an upper respiratory infection, and may be accompanied by aching, headache, epistaxis, and pleuritic chest pain. Since fever is often associated with these attacks of pain, and leukocytosis is a characteristic part of the picture of this hemolytic type of anemia, the similarity to an acute inflammatory process is quite striking. The joints may be somewhat swollen and warm and the symptoms migratory. The condition has often been mistaken for rheumatic fever because of the peculiar manifestations relating to the heart. Even during quiescent states when no pain or fever was present, these patients have in error been regarded as having chronic valvular heart disease, particularly mitral stenosis. The first such case that came to my attention was that of a young Negro girl in whom the physical signs relating to the heart were so striking that several clinicians stated most emphatically that she must have rheumatic heart disease with mitral insufficiency and stenosis.²¹ At autopsy only a diffusely enlarged heart with normal valves and pericardium was found.

Cardiac enlargement has been observed in at least 76 per cent of cases of sickle cell anemia.²² The heart is enlarged both on the right and on the left, it may be globular in shape, or the pulmonary conus may be very prominent.²³ Cardiac murmurs have been heard in at least 87 per cent of cases. The first sound at the apex is louder than normally, although it is not snapping in character. A systolic murmur, located best at the apex, is usually maximal early in systole. It may obscure the first heart sound. The second sound at the apex is often accentuated and a third heart sound is often heard early in diastole. Late in diastole there may be a murmur, and this presystolic murmur may blend with the first heart sound. A systolic murmur may be present in the pulmonic region and may be louder than the mitral murmur. The second pulmonic sound is usually accentuated and may be split. Diastolic murmurs at the base are rare.

In these patients pulsations may be prominent in the neck, the precordium is overactive—a phenomenon that is accentuated by a thin chest wall. A diffuse, wavy impulse may be readily visible in the fourth, fifth, and even sixth intercostal spaces to the left of the sternum. If the pulmonic conus is prominent, there may be a visible impulse and occasionally there is a bulge in the second and third interspaces to the left of the sternum. The point of maximum impulse is not well localized but is forceful and rolling, and there is a precordial lift. A diastolic tap may be felt in the

pulmonary area. A systolic thrill may be felt over the precordium and the vessels of the neck.²⁴ The electrocardiographic changes noted have been similar to those found in other types of anemia.

It is sometimes impossible to distinguish sickle cell anemia from acute rheumatic fever, or its cardiac manifestations from those of rheumatic heart disease. In Negroes the presence of sickle cell anemia should be suspected and the blood should be examined for sickle cells. If these are found, the probability is that one is dealing with sickle cell anemia rather than with rheumatic fever. It is noteworthy that in the former the red corpuscles often do not settle out rapidly in the sedimentation test, probably because of their abnormal shape. Furthermore, careful inquiry may reveal that the pains in the extremities are localized more in the bones than in the joints. Roentgenograms may reveal characteristic changes in the bones. Finally, salicylate therapy is usually of little value in sickle cell anemia.

Unlike other types of anemia, sickle cell anemia is extremely chronic, the patient often going about with a red cell count of less than 2 million and rarely of more than 3 million, with little variation for eight, ten, or even fifteen years. One may ask whether the striking cardiac manifestations of sickle cell anemia may not represent in an extreme degree an exaggeration of the cardiovascular adjustments to anemia that we have described as occurring in anemia in general.

Besides this possibility there is another. It has been suggested²⁵ that circulatory stasis in the small vessels of internal organs, muscles, and other tissues is the primary and the most perilous consequence of the sickle cell trait. Such stasis may be produced by the peculiar deformities of the red corpuscles and may put an added burden on the cardiovascular system. One of the many curious features of this disease is the extreme tortuosity of the blood vessels, which can often be observed in the ocular fundi.²³ This may be due to a congenital anomaly, one may also ask whether it could be caused by circulatory stasis. Cases of sickle cell anemia have been described in which numerous disseminated occlusions of the small pulmonary arteries were found and cor pulmonale was produced.²⁶ In these cases the small arteries were found to be lengthened, tortuous, hyalinized, and thrombotic—features revealing that these arteries had probably been subjected to severe, prolonged strain.

Whatever the mechanism may be, the heart in sickle cell anemia represents the extreme form of the heart in anemia.

SUMMARY

The symptoms and signs referable to the cardiovascular system that are associated with anemia are discussed, and the physiologic adjustments to anemia that take place in the cardiovascular system are considered. The capacity for adjustment when anemia develops gradually, appears to be very great. The remarkable changes found in the cardiovascular system in cases of sickle cell anemia may be the result of adjustments to severe anemia of exceptional chronicity.

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OBSERVATIONS ON THE EFFECT OF MASSIVE DOSES OF IRON GIVEN INTRAVENOUSLY TO PATIENTS WITH HYPOCHROMIC ANEMIA

By ANNE TOMPKINS GOETSCH, M D , CARL V MOORE, M D , AND
VIRGINIA MINNICH, M S

PARENTERAL administration of iron is impractical, dangerous, and unnecessary as a therapeutic procedure.^{1 2} It has, however, proved to be a valuable method for studying iron utilization and excretion both in animals and in human subjects with hypochromic microcytic anemia.

Polson³ and Cappell,⁴ for instance, observed that iron injected in rabbits, mice, and white rats was first taken up by cells of the reticulo-endothelial system but later redistributed in the liver, spleen, lymph nodes, and other tissues, where it remained for a period of months. Hahn and Whipple,^{5 6} Widdowson and McCance,^{7 7a} and Copp, Greenberg, and Cuthbertson⁸ made an important contribution to the understanding of iron metabolism by demonstrating that only small amounts of iron are eliminated in urine, bile, and feces after parenteral administration. In this way it was shown that the body has only a limited capacity for excreting iron except through hemorrhage.

Utilization of injected iron salts for hemoglobin synthesis has been studied in iron-deficient human subjects and in dogs. Whipple and Hahn^{6b} found that when from 108 to 216 mg of iron as colloidal ferric hydroxide was given intravenously during a two day period to dogs made anemic by induced chronic hemorrhage, only from 55 to 70 per cent of the total dose could be recovered in the liver and spleen twenty-four hours after the last injection. The tissues in which the remaining iron was deposited were not identified, but quantitative conversion of all of the injected iron to newly formed hemoglobin was observed in other animals that were given even larger doses of iron (700 mg) and not sacrificed for tissue studies.^{6a} Heath, Strauss, and Castle¹ gave daily intramuscular injections of 8, 16, or 32 mg of iron as iron citrate green for about ten days to 15 patients with hypochromic anemia. The resultant increase in total circulating hemoglobin indicated that an average of 96 per cent of the metal had been utilized for hemoglobin synthesis. Therapeutic effectiveness, however, was no greater for the 32 mg daily intramuscular injection than for a daily oral dose of 1000 mg of iron in the form of iron and ammonium citrate. For this reason, and because of the severe toxic reactions encountered, the authors concluded that it is undesirable to give iron parenterally. Fowler and Barer⁹ obtained a somewhat different result when they gave 100 mg of iron citrate green daily by intramuscular injection until from 139.7 to 304.8 mg of elemental iron had been injected in 4 patients with hypochromic anemia. Because no hemoglobin regeneration was observed, and because subsequent oral administration of iron was followed by more rapid hemoglobin regeneration than usually occurred in their patients, the authors postulated that the injected iron might have

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been used to restore depleted tissue iron. Early clinical experience with parenterally administered iron has been summarized by Stockman.¹⁰

The investigations described in this report were undertaken in an attempt to determine the maximum hematologic response that could be induced by iron therapy in patients with hypochromic anemia. It was felt that parenteral administration of an amount of iron sufficient to restore the circulating hemoglobin of iron-deficient subjects to normal would probably constitute a maximal stimulus to hemoglobin synthesis. Accordingly, this amount (from 0.608 to 1.32 Gm.) of elemental iron was given intravenously in one injection either as colloidal ferric hydroxide or as colloidal ferric oxide to each of 8 patients with hypochromic anemia. The reticulocyte response, rate of hemoglobin regeneration, and the percentage of utilization were followed. Three subjects without anemia were used for control observations.

Since comparably large amounts of iron have not previously been injected in human subjects, careful preliminary study was made to demonstrate that the infusions could be tolerated. It was determined that the lethal dose of intravenously administered colloidal ferric hydroxide for dogs was much greater than that of simple solutions of salts like ferrous chloride. This seemed a logical result, because the colloidal preparation is removed rapidly from the blood stream, presumably by the reticulo-endothelial cells, while soluble iron salts in doses of 1 mg. or more per kilogram of body weight require twelve hours or more for removal.¹¹ Hahn and Whipple¹² furthermore reported no toxic reactions following daily intravenous administration of 50 mg. of iron as colloidal ferric hydroxide to dogs until 700 mg. had been given. We initially injected 32 mg. of iron, and when it was found that this dose was tolerated by several patients, the amount given was increased to 100 mg. and then by several additional increments to 400 mg. The larger doses used in this study were tried only after the above mentioned preliminary observations had been completed.

Each of the 8 patients had a reticulocyte response that was significantly greater than is usual after optimal oral therapy. Since no rise in reticulocytes developed in the control subjects, the reticulocytosis cannot be regarded as nonspecific. The daily rise in hemoglobin averaged 0.224 Gm. per 100 cc., the lowest regeneration rate observed was 0.16 Gm. per day and the highest 0.27 Gm. It was calculated that from 72 to 99 per cent of the injected iron was utilized for hemoglobin formation. All of the patients improved in a dramatic manner. The toxic reactions during and immediately following the injections of iron, however, were severe and often alarming. In 3 other patients, administration had to be discontinued after 70, 123, and 180 mg. respectively had been given, because the systolic blood pressure fell to 60 mm. of mercury or less and signs of peripheral vascular collapse developed. All of the patients except 2 had distressing reactions. The toxic effects of parenterally administered iron were certainly severe enough to preclude its use for therapeutic purposes except under most unusual circumstances.

METHODS

Red blood cell counts, reticulocyte counts, and hemoglobin determinations were made daily on capillary blood. The Trenner red blood cell pipets and the counting

chambers were certified by the United States Bureau of Standards. Hemoglobin was measured by the oxyhemoglobin method described by Evelyn.¹² Both wet and dry preparations¹³ were used for reticulocyte counts, and the results obtained by the two methods were averaged. At intervals of from two to five days, venous blood was drawn for determination of packed red cell volume¹³ and of iron in serum.¹⁴ Blood volume was measured in 8 cases by the Evans blue dye technic.¹⁵

Eleven patients with hypochromic anemia were selected, each with a mean corpuscular hemoglobin concentration of from 21 to 29 per cent. The lowest initial red blood cell count was 2.70 million per cubic millimeter, the highest, 4.69 million, the average, 3.59 million. Initial hemoglobin values averaged 6.5 Gm. per 100 cc., with a range from 3.8 to 9.1 Gm. (table 1). In 10 of the 11 patients, the anemia was caused by chronic blood loss attributable in 8 cases to menorrhagia and in 2 to bleeding from the gastro-intestinal tract. The remaining patient was a young woman, 25 years of age, who had physical signs of hypogonadism (case 4). Her menarche had not appeared until she was 17 years old, her menstrual flow had always been scanty, and amenorrhea had developed one month before her admission to the hospital. No other cause of blood loss could be found, but her diet had been inadequate. Three subjects who had no anemia, no evidence of blood loss, and no hematologic abnormalities were used as controls.

After a control period of at least five days and in some instances several weeks, each of the patients was given slowly, by intravenous drip, a single injection of colloidal ferric hydroxide* (cases 1-6, 8, 12-14) or of colloidal ferric oxide† (cases 7, 9-11). The former preparation contained 3.2 mg. of elemental iron per cubic centimeter (pH 3.8) and the latter 3.5 mg. per cubic centimeter (pH 3.5). From 0.608 to 1.32 Gm. of iron was given, the dose was calculated in each instance to provide enough iron to raise the hemoglobin level from its initial value to one that approximated the normal for the individual. For this calculation, the determined blood volume was used in all instances but two (cases 4, 9), in which it was estimated to be 80 cc. per kilogram of body weight. The hemoglobin was assumed to contain 0.334 per cent of iron. The three control subjects were arbitrarily given 0.8, 1.008, and 1.008 Gm. of iron. Injection was made at the rate of from 10 to 25 drops per minute, so that a period of from two to five hours was required for the administration. Pulse rate, respiratory rate, and blood pressure readings were obtained at least once every fifteen minutes, and the patients were observed carefully for any evidences of toxic effect.

RESULTS

A. Toxic Manifestations during and immediately after Iron Infusion—Administration of a massive dose of iron was attempted fifteen times but was discontinued in three patients (cases 9, 10, 11) because of toxic manifestations. The twelve successfully completed infusions were given, one to each of 7 patients and 3 control subjects, and two with an interval of four months to 1 patient (case 3-a, b). All of the subjects except 2 (cases 4, 12) developed reactions during the injection, and even these

* Obtained through the courtesy of the William S. Merrell Company, Cincinnati.

† Obtained through the courtesy of John Wyeth and Company, Philadelphia.

TABLE 1.—Hematologic Response to Colloidal Iron Given Intravenously to 14 Patients

Case	Patient	Age	Sex	Cause of Anemia	Blood Volume (Cc)	Amt Elemental Iron Injected* (Gm)	Day of Therapy	R B C (Ml)	Hb (Gm)	Retic (%)	C V (%)	Serum Iron (Mg %)	Average Daily Rate of Hemoglobin Regeneration per 100 Cc of Blood (Gm)	Percent age of Utilization	Comment
1	E R	43	F	menorrhagia	4870	1 008	0 5 14 20	2 99 3 41 4 09 3 99	6 1 7 0 10 1 11 3	2 7 24 7 9 3 6 7	25 0 33 0		0 27	80 7	
2	A D	38	F	menorrhagia	3700	0 755	0 8 15 26	4 69 5 16 5 31 5 65	8 5 10 0 11 5 12 9	2 3 14 1 4 7 2 1	29 0 32 0 39 0 44 0		0 215	72 9	
3a	H B	24	F	gastro-intestinal bleeding	3780	1 024	0 9 33	3 02 3 50 4 03	3 8 5 4 9 0	6 2 22 1 3 7	18 5 24 0 33 0	0 009 1 300† 0 015 0 071	0 25	71 8	stools intermittently positive for occult blood
b		24	F	bleeding	3780	1 024	0 9 17 24 30	4 05 3 78 4 00 4 36 2 55	5 9 6 2 7 8 10 1 5 4	5 7 21 0 9 0 4 6 7 4	23 0 3 260† 0 075 0 068 35 0	0 017 3 260† 0 075 0 068 0 043	0 18	55 1	massive epistaxis and hematemesis at this point
4	A S	25	F	?	3000 (est)	0 608	0 9 23 98	3 36 3 78 4 08 4 63	7 0 9 4 11 3 11 3	5 0 18 3 2 9 2 0	24 0 34 0 34 0 36 0	0 011 0 612† 0 031 0 103 0 050	0 24	76 6	amenorrhea, no evidence of blood loss inadequate diet
5	K M	37	F	menorrhagia bleeding hemorthoids	4230	0 720	0 11 23 51	3 91 4 35 4 32 4 52	8 9 10 0 11 3 13 0	2 8 14 2 2 8 1 8	31 0 35 0 41 0	0 036 1 550 0 091 0 051	0 16	88 0	one profuse and one normal menstrual period during postinjection period
6	J L	47	M	gastro-intestinal hemorrhage	6800	1 320	0 10 18 47	4 26 4 37 5 50 5 04	9 1 10 6 13 1 13 6	4 5 16 3 4 3 1 6	32 0 35 0 44 0	0 003 3 860† 0 146 0 086 0 069	0 27	99 7	

7	A R	40	F	menorrhagia	4820	1 110	0	3 73	6 4	3 9	24 0	0 028 3 450† 0 163 128 0 199	0 21	76 8	profuse menstrual flow for 4 days moderately profuse menstrual flow for 5 days
8	M F	36	F	menorrhagia	3420	0 892	0	2 70	4 1	2 2	19 0	0 035 1 599† 0 035			patient left hospital on fifth day, rejected further observation
9	H B	37	F	menorrhagia		0 180	0	3 59 3 86 7 2 12 4	5 2 5 3 6 6 7 8	4 7 10 2 4 2	27 0 30 0 31 0				injection discontinued because of severe reaction
10	M G	41	F	menorrhagia	4230	0 123	0	3 27 2 80 3 31	5 3 5 4 6 1	3 9 16 6 2 9	19 0 21 0 28 0	0 032 0 407† 0 049 0 036			injection discontinued because of severe reaction
11	D H	?	F	menorrhagia	5030	0 070	0	2 57 2 29 8	4 9 5 2 5 3	5 1 11 1 8 4	18 0 22 0 20 0	0 027			injection discontinued because of severe reaction
12	M M	41	M	(control)		0 800	0	4 64 4 66 4 90	13 4 13 6 13 6	2 2 4 3 3 5	41 0 42 0 43 0				medical diagnosis psychoneurosis
13	E F	46	M	(control)		1 008	0	4 88	14 4	1 4	42 5	0 192 2 370† 0 158 0 137			medical diagnosis lateral sclerosis
14	V N	28	F	(control)		1 008	0	4 69 4 76 4 97	15 4 15 5 15 3	1 7 3 6 2 7	41 5 3 6 2 7	0 079 2 848 0 140 0 164			medical diagnosis Parkinson's syndrome

* Iron injected as colloidal ferric hydroxide in patients 1-6, 9, and control patients 12-14, as colloidal ferric oxide in patients 7, 8, 10, 11

† Serum drawn for serum iron immediately after completion of injection

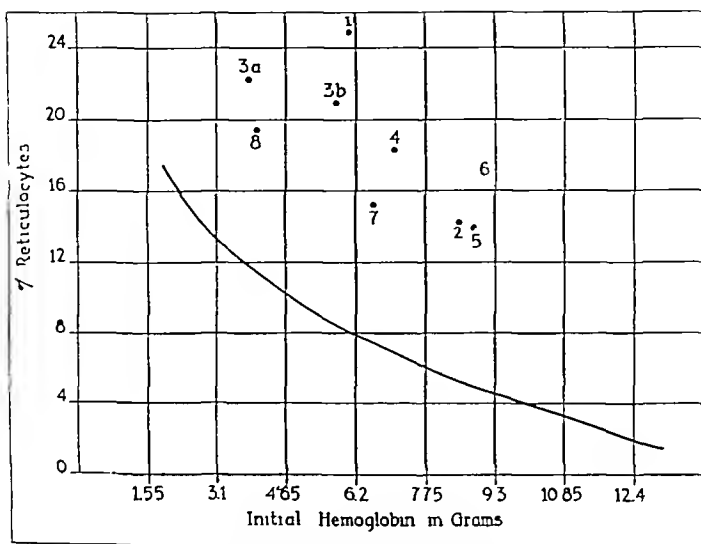
two persons had fever and nausea several hours later. In general, the reactions were similar to those described by Heath, Castle, and Strauss,¹ but apparently more severe. Within a few minutes after the intravenous drip was started, patients noted nasal stuffiness, a desire to sneeze, lacrimation, paresthesias, flushing of the face, and, less frequently, palpitation and pounding headache. They often complained of sensations of swelling and stiffness of the tongue and face. The paresthesias consisted of burning or tingling, usually in the tongue and hands, but sometimes involving the whole body. Nausea occurred with fourteen of the fifteen injections, in half the instances it appeared from one to two hours after the administration had been completed. In the more severe reactions, vomiting, diarrhea, abdominal or low back pain, and transient fall in blood pressure developed. These manifestations could not be prevented by administration of 0.0006 Gm. of atropine, but almost always disappeared when the rate of flow was decreased. The fall in systolic pressure was usually not greater than from 20 to 30 mm. of mercury, but in three instances was alarming (to 60 mm. of mercury in two instances and to 30 mm. in the third). These latter 3 subjects were the ones in whom the injection was stopped, they also had severe abdominal pain, low back pain, vomiting, and diarrhea. Tachycardia was observed ten times, sensation of substernal oppression twice.

Venous blood withdrawn immediately after the iron had all been given showed that a variable amount of hemoconcentration had occurred (packed red cell volume increased from 10 to 20 per cent), but gave no evidence of hemoglobinemia. All the subjects, including the 3 controls, developed fever shortly after the injection had been finished, in twelve instances, it was associated with a chill. Rectal temperatures rose to between 38 and 39.5 C. but returned to normal within from six to eight hours. The vein into which the iron was injected always became thrombosed.

On the day following the injection most of the patients complained of a profound sensation of weakness. Within forty-eight hours, however, this had entirely disappeared and their appetites were as dramatically improved as are those of patients with Addisonian pernicious anemia after liver therapy. Two subjects developed severe pleural pain after twenty-four and forty-eight hours respectively, no friction rub could be heard and there was neither clinical nor roentgenologic evidence of pulmonary infarct or consolidation. These symptoms disappeared slowly. No other late reactions of any kind were observed.

The pharmacologic explanation for these reactions is not clear. The changes produced by the two different preparations used were qualitatively and quantitatively similar. They resembled nitritoid reactions and speed shock.¹⁸ That the latter probably does not provide the explanation in this instance is suggested, since (1) the severity of symptoms did not always parallel the speed of injection, and (2) the toxic manifestations were similar to those observed when much smaller amounts of iron are given intramuscularly.¹ They are probably evidences of a heavy metal effect.

B Reticulocyte Response The reticulocytes usually began to increase within forty-eight hours after the injection and reached the peak values of their response in from five to eleven days. The highest value observed was 24.7 per cent (case 1). The increase was significantly greater in every instance than is the usual response to oral



* Number at each point indicates case number

FIGURE 1 RETICULOCYTE PEAKS FOLLOWING SINGLE LARGE INJECTION OF COLLOIDAL FERRIC HYDROXIDE AND FERRIC OXIDE

(Plotted on Heath's Curve of Adequate Response 18)

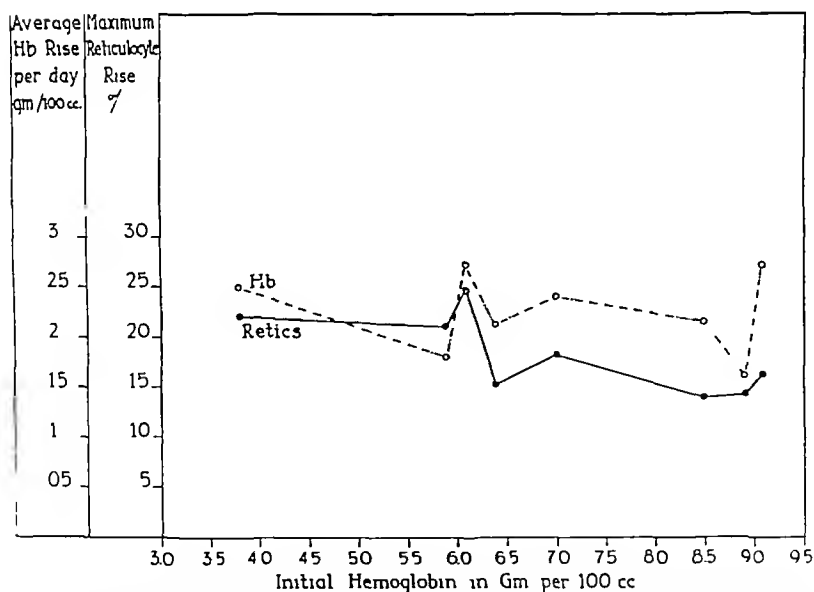


FIGURE 2. COMPARISON BETWEEN INITIAL HEMOGLOBIN LEVEL, MAXIMUM RETICULOCYTE RISE, AND RATE OF HEMOGLOBIN REGENERATION IN SUBJECTS WITH HYPOCHROMIC MICROCYTIC ANEMIA FOLLOWING MASSIVE DOSES OF IRON GIVEN INTRAVENOUSLY

iron therapy. This is well demonstrated in figure 1, in which the maximum levels are charted against the values given by Minor and Heath^{17, 18} for the reticulocyt-

sis to be expected after optimal oral therapy. Minor and Heath found that the height of the reticulocyte rise when iron is given orally to patients with hypochromic microcytic anemia is, in general, inversely proportional to the initial erythrocyte and hemoglobin levels. They observed, however, that the relationships are less constant than in pernicious anemia after liver therapy. From table 1 and figure 2 it can be seen that there was no correlation in our nine instances of parenteral iron therapy between the initial hemoglobin and the reticulocyte peak. The number of cases is too small to permit any conclusions to be drawn, the observation is merely recorded.

No reticulocytosis occurred when comparably large amounts of colloidal iron preparations were given to the 3 control subjects (cases 12-14). The response, therefore, was not nonspecific.

Of special interest are the reticulocyte rises observed in the 3 patients who received only moderate doses of iron because of the interruption of the injection. A reticulocyte peak of 10.2 per cent in patient 9 followed the injection of 180 mg of iron, a rise to 16.6 per cent occurred in patient 10, who received 123 mg, a rise to 11.1 per cent was observed in patient 11, who received 70 mg. It is apparent, when these figures are compared with Heath's curve, that 70 mg of iron given parenterally can cause a reticulocyte response as great as would be expected following optimal oral therapy. In 1 patient (case 10), 0.8 Gm of ferrous sulfate was given orally each day, beginning on the twenty-second day after the small parenteral dose of iron, a second reticulocyte rise to 13 per cent occurred.

C. Rate of Hemoglobin Regeneration—The hemoglobin level in most instances began to rise within three or four days after the iron had been injected, increased rapidly during the subsequent two weeks, and then rose more gradually as the values approached the normal for the individual subject. This is well illustrated by the results obtained in case 6, which are graphically recorded in figure 3. This man's hemoglobin increased at a rate of 0.27 Gm per 100 cc of blood per day. In other patients, values from 0.16 to 0.27 Gm were obtained. The average rate was 0.224 Gm. These figures are based on eight complete periods of observation in seven patients. The value for the eighth patient in the series is not included because she failed to return for observation after the first week. There was no direct correlation between the initial hemoglobin and the rapidity of hemoglobin regeneration (fig. 2). In the two patients who showed the most rapid response (cases 1, 6), the initial hemoglobin levels were 6.1 and 9.1 Gm, with erythrocyte counts of 2.99 and 4.26 million cells per cubic millimeter. The lowest rate of regeneration was seen in case 5, in which the initial hemoglobin was 8.9 Gm and the red blood cell count 3.91 million. In several of the patients (cases 3, 5, 7) the figures obtained for the daily rise in hemoglobin were probably lower than the true values, because hemorrhage occurred during the period of regeneration. However, since a maximal (or nearly maximal) stimulus to hemoglobin synthesis was used in these patients, it seems fair to assume that the observed rates of regeneration were also nearly maximal. The assumption is supported by the fact that hemoglobin increases at about the same rate after hemorrhage, or after liver therapy in patients with pernicious anemia.

Observations made by other investigators of therapeutically induced hemoglobin

regeneration in patients with iron deficiency anemia are summarized in table 2. These data indicate that in general the rate of synthesis was greater with the lower initial levels of hemoglobin. Even so, with oral therapy, the most rapid rates of increase were slightly above 0.2 Gm per day, although Reznikoff stated that he had observed an occasional patient in whom the increase was as rapid as 0.29 Gm per 100 cc per day. This is certainly unusual, however, and the great majority of the figures fall below 0.2 Gm per day. Data for the response to parenteral therapy are much more meager, but Heath, Castle, and Strauss¹ found a rate of 0.296 Gm

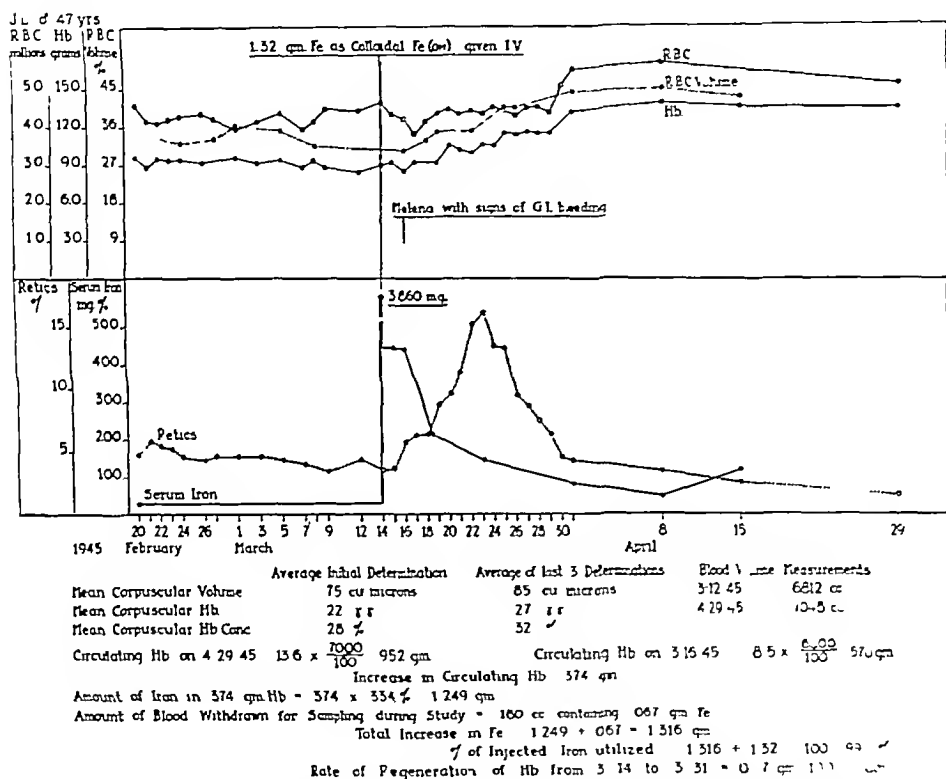


FIGURE 3 THERAPEUTIC RESPONSE OF A PATIENT WITH HYPOCHROMIC MICROCYTIC ANEMIA TO ONE LARGE DOSE OF COLLOIDAL FERRIC HYDROXIDE GIVEN INTRAVENOUSLY

per 100 cc per day in 3 subjects who had been given 0.32 Gm of iron daily, in the form of iron citrate green, by intramuscular injection. Attention is called to the fact that the figures summarized in table 2 are not strictly comparable. The rate of hemoglobin rise slows as the hemoglobin value approaches normal. The actual period of time used for calculating the average daily increase after therapy has been started may alter the value considerably. This period varies in different reports and many authors do not give the data at all.

D Utilization of Injected Iron—Utilization of the injected iron as calculated from the determined (seven times) or estimated (one time) blood volume showed an

Friend, D G ²¹	iron ascorbate	0 200 (metallic iron)	15 3	not stated < 7 8	0 170 0 232	
Moore, C V, Minnich V, Vilter R W, Spies, T D ³	ferrous sulfate	0 8	8	5 9-8 9	0 145	response altered by profuse menstrual blood loss during therapy
Reznikoff, Paul ²⁰	several	varied	not stated	< 7 25 > 7 25	0 145 0 072	daily Hb regeneration is high as 290 Gm / 100 cc / day in occasional case
Schiff, E ²⁷	ferrous lactate or ferrous tartrate	1 5	9 7	< 5 52 5 52-6 9	0 138-0 22 0 042-0 14	

B PARENTERAL THERAPY

Heath, C W, Strauss, M, Castle, W B ¹	colloidal ferric hydroxide or citrate green citrate green citrate green	(metallic iron) 0 008 0 016 0 032	5 2 3	3 4-5 6 3 5-5 3 3 0-5 6	0 047 0 068 0 296	
Reznikoff, Paul, Goebel, W F ²²	ferrous gluconate	0 156-0 475†	4	4 6-10 2	0 189	no absolute correlation with initial hemoglobin level
Friend, D G ¹	iron ascorbate	0 010	3	not stated	0 186	
This report	colloidal ferric hydroxide or colloidal ferric oxide	0 608-1 32 Gm iron in a single dose	8	8	0 224	

* In terms of preparation given unless otherwise stated

† The daily rise in Hb decreases as the hemoglobin level approaches normal, but very few authors state the number of days of therapy used in determining this figure

‡ This value represents the total dose of iron given

average utilization of 80.8 per cent, with variation of from 71.8 to 99.7 per cent. The result obtained following the second administration in case 3 (b) is not included in this average, because the patient had a massive hematemesis and epistaxis during the regeneration period. As was the case with the rates of hemoglobin rise, these figures are lower than they should have been, because hemorrhage occurred in cases 3, 5, and 7. They are, furthermore, subject to the influence of whatever errors are inherent in the determination or estimation of blood volume. Nevertheless, the results confirm observations of other workers to the effect both that parenterally administered iron is almost completely retained by the body,⁵⁻⁹ and that it is quantitatively converted into hemoglobin.^{1, 5, 6} In 3 of Heath's cases,¹ the percentage of utilization was less than 70, his average value was 96 per cent, because the figures for several other patients were above 100 per cent.

E *Changes in Serum Iron Values*.—Serum iron determined in seven instances immediately after the iron injections ranged from 0.622 to 3.86 mg. per 100 cc. This last figure is amazingly high and has never before been recorded for a human subject. The values remained elevated after twenty-four hours but subsequently fell to levels below the normal range in 3 subjects (cases 3-a, 4, 8) and to normal in 4 (cases 3-b, 5, 6, 7). This latter result was not unexpected, since the body's deficiency of iron was at least largely corrected by the infusion. Initial serum iron values were always low.

SUMMARY AND CONCLUSIONS

Massive doses of iron (from 0.608 to 1.32 Gm. as colloidal ferric hydroxide or colloidal ferric oxide) were given intravenously in single infusions to 8 different patients with hypochromic microcytic anemia. One patient was given a second injection after an interval of four months, so that nine administrations were made. The following observations were made:

1. The reticulocyte response was higher in each instance than would be expected in oral therapy. In 3 additional patients in whom injection had to be discontinued after 0.070, 0.180, and 0.123 Gm. of elemental iron had respectively been given, the reticulocyte rises were higher than were the average responses reported by Heath^{1, 5} after optimal oral therapy. This at least suggests that optimal oral therapy does not provide a maximal stimulus to outpouring of reticulocytes from the bone marrow. Comparable doses of iron given to 3 control subjects with normal hemoglobin levels did not cause a reticulocytosis.

2. The average rate of hemoglobin regeneration per 100 cc. of blood per day was 0.224 Gm., the lowest value was 0.16 Gm. and the highest 0.27 Gm. These figures were calculated for the rise that occurred from the day of iron administration to the time at which the rate of hemoglobin increase was obviously becoming slower. Since correction was not made for blood loss in 3 of the patients during the period of regeneration, the figures for the rate of hemoglobin formation are lower than they otherwise would have been. Even so they are distinctly greater than those usually obtained following oral therapy (table 2), but no greater than is found in an occasional patient given iron by mouth. The data suggest that the fastest rate of hemoglobin regeneration that can be stimulated by iron in subjects with hypochromic anemia approximates 0.3 Gm. per 100 cc. per day.

3 Calculations indicated that from 71.8 to 99.7 per cent of the injected iron was apparently used for the synthesis of hemoglobin. These figures are likewise lower than they would have been if several of the patients had not lost blood during the recovery period. The observation of other workers that parenterally administered iron is almost completely retained by the body and converted into hemoglobin was therefore confirmed.

4 Toxic reactions to the injected iron are described in detail. They were severe in all but two instances, and in 3 patients were so alarming that injection of iron had to be discontinued. There can be no doubt that the reactions to iron parenterally administered in large doses are great enough to contra-indicate use of this measure as a therapeutic procedure.

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ERYTHROBLASTOSIS FETALIS IN THE FIRST-BORN

PREVENTION OF ITS MOST SEVERE FORMS

By PHILIP LEVINE, M D , AND R K WALLER, M D

AS a result of our knowledge of the pathogenesis of erythroblastosis fetalis, a new diagnostic procedure, testing for the Rh factor, has been made available^{1 2} Another outcome of the early work was the recommendation³ that human anti-Rh agglutinins derived from mothers of erythroblastotic infants are far preferable to experimental anti-Rh blood immune serum, which, by and large, is now no longer used * Tests with potent diagnostic anti-Rh serums were recommended for all women with a history of fetal or neonatal morbidity, in order to prevent intragroup transfusion accidents, and for the selection of Rh negative blood for the affected infants of Rh negative mothers The findings presented below indicate that future emphasis should be placed on the prevention of iso-immunization by transfusions in the group of Rh negative female patients at *any* time prior to possible pregnancies

Iso-immunization by the Rh factor occurs in two groups of cases, namely, (1) Rh negative individuals after repeated transfusions of Rh positive blood, (2) Rh negative women immunized by Rh positive fetal blood A third group may be mentioned in which both factors, transfusions and pregnancy, are operative

This paper deals mainly with selected cases of the third group in which iso-immunization was initiated by transfusions and, after a variable interval, became intensified through pregnancies Evidence is presented which indicates that the combined action of both factors in the order given served to increase the number of infants with erythroblastosis fetalis as well as the severity of the condition in the infant †

It is now generally accepted that 92 per cent of all cases of erythroblastosis fetalis result from (1) immunization of the Rh negative mother by Rh positive fetal blood, and (2) subsequent action of maternal anti-Rh antibodies (agglutinins and blocking antibodies) on the susceptible Rh positive fetal blood The obstetric histories of mothers of erythroblastotic infants reveal that this condition occurs in the first-born only very rarely Obviously, one or more pregnancies with Rh positive infants are required to induce a sufficient degree of iso-immunization Once an Rh negative woman is immunized, each successive pregnancy with an Rh positive fetus results in increasingly severe forms of erythroblastosis fetalis ‡

From the laboratories of the Ortho Research Foundation, Linden N J

* In the statistical studies on the pathogenesis of erythroblastosis fetalis, human anti Rh serums were used exclusively

† For a preliminary report, cf Levine⁴

‡ In this paper the terms Rh positive and Rh negative refer to reactions with the diagnostic serum of choice, i e , anti Rh₀ which detects about 92 per cent of all instances of iso-immunization by the Rh factor It is not advisable for the clinician to commit to memory several terminologies based on the action

The observation in 1944 of a young Rh negative woman, aged 20 (case 1, table 2), whose first pregnancy resulted in the most severe form of erythroblastosis fetalis—fetal hydrops—suggested the possibility that the patient was already immunized at the time of her first pregnancy. On being questioned, she stated that at the age of 6 (fourteen years before) she had been transfused several times with her father's blood. Although immune antibodies (anti-Rh agglutinins or blocking antibodies), in contrast to normal iso-antibodies, have a comparatively short duration in the blood plasma—i.e., from several weeks to one or more years after pregnancy—the reticulo-endothelial cells responsible for antibody production probably retain the sensitized state for a long time, if not permanently.⁶ Under such conditions, the response to readministration of the same antigen even many years later, in the form of either fetal blood or transfusion, is more rapid and more intense. These cases serve as excellent examples of the phenomenon known to immunologists as the anamnestic reaction.

The anamnestic reaction represents the production in response to an antigenic stimulus of an antibody that has been produced in the tissues on some previous occasion.⁷ In the past, most workers were interested mainly in nonspecific response, but the term is used here to indicate a response to restimulation many years later by the same antigen, i.e., the Rh factor. In 3 cases published by Levine,⁶ the immunization in Rh negative patients was initiated by pregnancy, and many years later transfusion of Rh positive blood resulted most unexpectedly in severe reactions with varying degrees of oliguria. Cases of a similar nature were also observed by Weiss.⁸

In one of the cases cited an Rh negative woman born in 1885, had one daughter (Rh positive) in 1904. There were no other pregnancies. In 1937, the patient had one uneventful blood transfusion from her Rh positive daughter, given because of mastoiditis. This additional stimulus apparently immunized the patient, so that in May 1941, four years later, when a diagnosis of leukemia was established, another transfusion of her daughter's blood was followed by a delayed reaction.* A further transfusion from another Rh positive donor three months later resulted in a severe reaction and oliguria. Anti Rh agglutinins of moderate activity were found.

Accordingly, the possibility suggested itself that erythroblastosis fetalis in the first-born might occur far more frequently if the Rh negative patient had been transfused at any time prior to the pregnancy, even in her childhood or girlhood. This view could be put to the test in a statistical analysis of the large material tested in the years from 1940 to 1945.

In this study, limited to approximately 700 Rh negative women, only twenty-eight instances were found in which the first Rh positive infant had erythroblastosis fetalis.† Of these, nineteen mothers gave histories of one or more transfusions

of other varieties of anti Rh serums that are either important in very few cases or not available for distribution. It is, however, necessary for the clinician to be guided by consulting with those serologists who conduct active research in this field.⁵

* For a description of a similar case with a delayed reaction to a transfusion cf. Levine et al.⁶

† The incidence of erythroblastosis fetalis in the first born cannot be derived from this study, since complete obstetric histories were not always available.

at various times prior to the first full term or almost full term pregnancy, and no such history could be elicited in a control group of nine mothers. By itself, the ratio of 2:1 was disappointing, because it was felt that the effects of previous iso-immunization by transfusion would be far more striking. Further reference to the possible role of other immunizing stimuli in the control group will be made below. In any event, the true state of affairs was revealed by taking into account the severity of the condition in the infant. These findings are summarized in table 1.

These findings seemed to justify the conclusion that previous transfusions initiate iso-immunization, which is intensified by the first pregnancy.* In nine of eleven subsequent pregnancies, the end result was fetal death, while two women are still to be delivered. The most severe forms, ending in intra-uterine fetal death, are twelve times as frequent as mild cases.† Assuming that all severe cases can be successfully treated with transfusions of Rh negative blood and by other measures, only 7 of the 19 cases could have been saved, in contrast to 8 of 9 cases in the control group.

TABLE 1—*Erythroblastosis Fetalis in the First Rh+ Infant in Rh- Women*

	Transfusion History	
	Positive	Negative
No cases	19	9
Severity of disease		
mild	1	4
severe	6	4
fetal death	12	1

The single death in the control group may possibly be attributed to causes other than erythroblastosis fetalis. The blood of this patient, Mrs. Mar (case 25, table 4), group O, Rh negative, was submitted by Dr. Lowell Erf. The patient was 24 years old, and her first pregnancy was to terminate in the middle of November 1944. However, she failed to develop spontaneous labor. On Dec. 4 fetal heart sounds were no longer heard, and a medical induction was started. After much difficulty, a yellow, macerated fetus was expelled. Microscopic sections revealed that the bone marrow was hyperplastic and that there was some extra-medullary hematopoiesis in the spleen and liver. The mother developed septicemia subsequent to a pelvic peritonitis and expired Dec. 12. Tests of the mother's blood three days prior to her death were negative for agglutinins and blocking antibodies.

The essential details in the cases of the nineteen Rh negative mothers previously immunized by transfusions are set forth in table 2.

Of the nineteen Rh negative women, three (cases 10, 16, 19) had early pregnancies prior to the first full term or almost full term pregnancy with an Rh positive infant. Two of these had early abortions, while the third had a ruptured ectopic gestation. These cases can be included in the series, since iso-immunization cannot be initiated by fetal blood unless the pregnancy is so well advanced that the placenta presents a sufficiently large surface area of fetal villi to the maternal

* On a theoretic basis, this possibility has already been mentioned by Katzin¹⁰ and Mollinson.¹¹

† A high incidence of fetal death was also reported by Diamond¹² in a similar group of cases.

TABLE 2

Case No.	Patient (Mother)	Physician	Interval between transfusion and pregnancy (yr)	No. transfusions	Indication for transfusion	Condition of first Rh+ baby	Subsequent pregnancies	Rh of father	Anti Rh Antibodies in Mother		Remarks
									Agglutinations	Blocking	
1	Dw	Auerbach Irvington N J	11	1	measles scarlet fever	fetal hydrops (1914)		Rh ₂	negative		not tested for blocking anti bodies all 9 transfusions over period of 65 days & uneventful not tested for blocking anti bodies
2	Cw	J Dickson Nashville Tenn	1	0	ovarian cyst pelvic adhesions	severe 1 transfusions death (1912)		Rh1/Rh ₂	negative		
3	Mc	C A Dille Dayton O	6	1	bleeding after tonsillectomy	fetal death (1912)	1944 stillbirth	Rh1/Rh ₂	weak		
4	T	W Tirman Brooklyn	10	2	mastoidectomy	fetal death at full term (1913)	now pregnant	Rh ₁	negative		during 1911 pregnancy re ceived multiple subcutane ous injections of husband's blood for desensitization not tested for blocking anti bodies
5	Rf	C Kinsey Staten Island N Y	5	1	menorrhagia	fetal death at full term (1939)	1941 fetal death at 34 wk	Rh1/Rh ₂	weak (1944)		
6	ko	L R Pyle Brooklyn	2	1	bleeding after tonsillectomy (250 cc)	fetal death at 6 mo (1942)	1943 fetal death at 28 wk	Rh1/Rh ₂	strong		
7	Do	H H Ware Richmond Va	11	several	pseudoleiomyphilia	severe several trans fusions, recovery (1914)	1943 fetal death at 36 wk	Rh1/Rh ₂	strong		baby's blood behaved like Rh probably Rh ₁ with partial blocking severe reaction with anuria following transfusion dur ing pregnancy later re ceived Rh- blood 1940-first pregnancy re sulted in normal Rh- child 2 transfusions with husband's blood during this pregnancy 1942-first pregnancy re sulted in early abortion 1944-second pregnancy 2 transfusions with Rh- blood Donor Rh ₂
8	Pl	H H Ware Richmond Va	4	several	injuries	mild not transfused (1942)	1945 hydrops at 34 wk	Rh1/Rh ₂ (homozygous)	strong (1945)		
9	Wat	R Johnston Houston Tex	2	3	pyelitis anemia	macerated fetus at full term (1942)	1944 fetal death at 31 wk	Rh ₁ r (heterozygous)	strong		
10	Li	R Johnston Houston Tex	8	1	fractured pelvis	fetal hydrops 7 mo (1941)			strong		baby's blood behaved like Rh- owing to action of blocking antibody
11	Ha	J Davenport New Orleans	9	1	septicemia	severe death (1941)	1945 fetal death at 28 wk	Rh1/Rh ₂	weak		
12	DeB	J Davenport New Orleans	2	8	ruptured appendix peritonitis	macerated fetus (1943)	now pregnant 2 mo	Rh ₂	weak (1945)	strong	
13	Haf	C V Herrman Kansas City Mo	12	2	orthopedic op- eration	severe recovery after transfusion of Rh- blood (1945)		Rh1/Rh ₂	strong		

14	Ham	P. I. Lebling New York	9	2	erythema thorax anemia	severe death (1945) moderately severe 1 transfusion (1943)		RhRh ₁		strong weak (1945)	transfused once more following delivery, with severe reaction
15	Ham	W. J. Mitchell Jr Newark, N. J.	2	1		fetal death at 33 wk (1945)		Rh ₀		strong	not immunized by early pregnancy 3 transfusions at operation uneventful
16	Sm	C. W. Schelm Battle Creek, Mich.	6	3	ectopic pregnancy (6 wk.)	severe several transfusions recovery (1944)	1945 fetal death at 36 wk	RhHr— (homozygous) Rh ₁ Hr— (homozygous)		strong	advised to terminate pregnancy but refused
17	Ne	A. F. Rokoff Philadelphia	2	1	duodenal ulcer	fetal death (1944)	1945 fetal death			distinct	
18	Ga	R. H. Stewart Seattle	1	1	middle ear infection					strong	not immunized by early pregnancy transfusion of his blood uneventful
19	Wa	M. Wachstein Middletown, N. Y.	1	1	bleeding after early abortion (1944)	macerated fetus (1945)					

sinuses.⁶ Furthermore, as the pregnancy progresses, the blood vessels in the villi become large and approach nearer to the maternal sinuses. That distinct iso-immunization was not induced in the early pregnancies was also indicated by the absence of untoward reactions in 2 of the 3 patients who required immediate blood transfusion.

It is significant that the intensity of the hemolytic process in the first Rh positive fetus was not influenced by the number of transfusions preceding the pregnancy (table 3).

A few of the cases listed above are selected for additional comment.

Patient Gv (case 2) was transfused nine times uneventfully in 1940 for ovarian cyst. In 1942, in her first pregnancy, she was delivered of an infant with severe anemia and jaundice. The infant was transfused four times but expired. There were no anti-Rh agglutinins in the mother's serum tested one month after the delivery but it may be assumed that blocking antibodies had been present.

Patient Mc (case 3), group O, Rh negative, was transfused once because of excessive bleeding following a tonsillectomy in 1936. Her first pregnancy, in 1942, ended in a full term stillbirth, fetal heart sounds having ceased in the latter part of the ninth month. In 1944 she was again pregnant and during the course of this pregnancy received a series of subcutaneous injections of her husband's blood for the purpose of desensitization. The end result was another stillbirth. When tested in May 1944 the patient

TABLE 3

Severity of Disease	Immunized by	
	1 transfusion	More than 1 transfusion
Mild	0	1
Severe	3	5
Fetal death	6	4
Totals	9	10

was found to be immunized (anti Rh agglutinins). Her husband was in group O, Rh positive (type Rh₁ Rh₂). It is quite evident that desensitization cannot be accomplished by injection of small amounts of Rh positive blood. It is more likely that these injections could only have the opposite effect of increasing the degree of iso-immunization.

Patient Wat (case 11), group O, Rh negative, was transfused uneventfully twice during her first pregnancy and once post partum. This case can be included in this series because the first child, born in 1940, was Rh negative and normal. The husband (group O, Rh₁, Hr positive) was used as donor twice. These transfusions immunized the patient so that two years later her second pregnancy—the first with an Rh positive fetus—resulted in intra uterine fetal death. A third pregnancy resulted in intra uterine fetal death at thirty-one weeks. Tests eleven months after the last pregnancy revealed the presence of strong blocking antibodies.

Patient Ha (case 11), 28 years old, group O, Rh negative, was transfused only once from her Rh positive uncle (Rh₂), in 1935. Her first pregnancy terminated in August 1944, and the infant had a severe form of erythroblastosis fetalis. This case is significant because only one transfusion, of 9 years before, was sufficient to initiate the iso-immunization. Tests of the patient's serum seven months after the delivery revealed the presence of weak anti Rh agglutinins.

Patient Sm (case 16) was transfused three times uneventfully in 1939 for an early ectopic pregnancy. In 1945, the first full term pregnancy was terminated by cesarean section at thirty-three weeks and a stillborn infant was delivered. The patient was immunized, as indicated by the presence of strong blocking antibodies. As already mentioned, this case can be included in the series because iso-immunization is hardly likely to occur in the presence of an early ectopic pregnancy.

Two patients, PI and Ham (cases 8-15) who were transfused during the state of active iso-immunization, suffered transfusion reactions and one of them (PI) had complete anuria, from which she finally recovered. This patient subsequently had several uneventful transfusions of Rh negative blood.

It was not always possible to test the blood of the affected infant, but evidence of iso-immunization could be obtained in all but three instances, and these were studied prior to the description of the tests for blocking antibodies.¹²⁻¹⁴ Judging from the high incidence of blocking antibodies in the remaining cases, it is safe to assume their existence in at least two of the three mothers who were tested soon after their deliveries.

In two instances, the affected infant's blood tested at birth failed to agglutinate with anti-Rh₀ serums, but their mothers (cases 7, 13) had strong blocking antibodies that rendered their blood inagglutinable with anti-Rh₀ serums. In case 7 the infant's blood reacted with anti-Rh' serums, so that the blood behaved as though it belonged to subtype Rh'.

Although histologic evidence to support a diagnosis of erythroblastosis fetalis was frequently not available, the selection of the cases can now be accepted on the basis of serologic findings in Rh negative mothers who have been immunized, as indicated by the presence of agglutinins or blocking antibodies. These serologic tests were of particular value in cases in which autopsies were not performed or in which the tissues were too much macerated to permit of histologic examination.

Of the sixteen fathers tested, three were homozygous for the Rh₁ factor, as indicated by a negative reaction with anti-Hr serums.¹⁵⁻³ Four fathers were Rh positive, of the subtype Rh₁Rh₂. Bloods of this type are heterozygous from a genetic viewpoint, but in matings of these men with Rh negative women all the offspring must be Rh positive, 50 per cent of the type Rh₂ and 50 per cent of the type Rh₁.¹⁶ To all intents and purposes, these seven mothers had been deprived of an opportunity of having normal offspring as a result of previous transfusion.

Of the remaining nine fathers, only one (case 9) is definitely of the heterozygous genotype, Rh₁rh, since the first child was Rh negative. The blood of four fathers was not tested with anti-Rh" serum, so that the outlook for future pregnancies in these four matings and in the four matings in which the father is of the type Rh₁Hr positive could not be determined.

The incidence of an additional incompatibility involving the blood factors A and B in this group of cases is 37.5 per cent, or almost identical with that found in the much larger group of Rh negative mothers of erythroblastotic infants (35 per cent).¹⁷

THE CONTROL GROUP (NOT PREVIOUSLY TRANSFUSED)

As indicated above, it was most surprising to find as many as nine instances of erythroblastosis fetalis in the first full term presumably Rh positive infants in the group of mothers not previously immunized by transfusions. The pertinent data in these cases are presented in outline form in table 4. A few of these cases are selected for brief comment.

Case 21. Mrs. Rei had four pregnancies in the years from 1941 to 1945. Her blood, tested at irregular intervals during the last three years, always contained anti-Rh agglutinins. Her first baby had mild

erythroblastosis fetalis that required no treatment. Her second child (1942) had severe hemolytic symptoms and in addition exhibited spasticity probably indicative of kernicterus. Her third child (1943) died after a few days of severe anemia and jaundice. At this time, Mrs. Rei was advised not to become pregnant again until her agglutinins had disappeared. Unfortunately, the patient soon became pregnant while moderately active antibodies were still present. Since her husband was homozygous for the Rh factor it was suggested that the circumstances were such as to justify a termination of the pregnancy which was carried out. It is quite probable that the intensity of the iso-immunization in cases of this sort

TABLE 4

Case No.	Patient (mother)	Physician	Condition of Rh+ Baby	Subsequent Pregnancies	Rh of Father	Anti Rh Antibodies in Mother		Remarks
						Agglutinins	Blocking	
20	De	J. N. Pannullo Newark, N. J.	severe lived 1 wk (1943)	now pregnant 1 mo	Rh ₊	negative		anti Rh in 1943
21	Re	R. Berman Newark, N. J.	mild (1941)	1942 severe kernicterus 1943 severe death 1945 early pregnancy	Rh ₁ Hr-	moderate		on recommendation pregnancy terminated never lost agglutinins
22	Ga	I. H. Erb Toronto Ont. Canada	severe death 2d day (1944)		Rh ₁ Hr-	negative	negative	father homozygous
23	Ro	R. E. Kelso Washington D. C.	mild (1940)	severe death in 6 days kernicterus	Rh ₁ Hr-	negative	negative	father homozygous
24	Sh	C. G. Hanson Cranford N. J.	mild (1944)		Rh ₁ Hr-	negative	negative	father homozygous
25	Mar	L. Erf Philadelphia	fetal death			negative	negative	not much evidence of erythroblastosis term over due congenital malformation (?)
26	No	G. V. Herrman Kansas City Mo.	severe Mongolian idiocy (1943)	1944 miscarriage 1945 normal Rh-	Rh ₁ Hr+	negative	negative	1942-miscarriage preceding first full term pregnancy father must be heterozygous
27	Ma	G. V. Herrman Kansas City Mo.	severe death (1943)	1945 severe jaundice transfusion	Rh ₁ Hr-	negative	moderate	mother transfused after first delivery chills and fever father homozygous
28	Pa	G. V. Herrman Kansas City Mo.	mild		Rh ₁	negative	negative	father not tested for Rh ⁺ or Hr

can be diminished by longer intervals between pregnancies. At any rate, in carefully selected cases, characterized by (1) history of several instances of fetal or neonatal morbidity, (2) active iso-immunization, and (3) a homozygous (clinically or genetically)¹⁸ husband, further pregnancies should not be encouraged. In the event that such patients accidentally become pregnant, there is already sufficient clinical evidence to justify termination of the pregnancy.

Case 26. Mrs. No had an early pregnancy that terminated in an abortion. In her first full term pregnancy, the infant had severe hemolytic disease as well as the syndrome known as Mongolian idiocy.

This was followed by a miscarriage in 1944 and delivery of a full term normal Rh negative infant. Accordingly, the husband is heterozygous for the Rh factor, and there is good outlook for future normal Rh negative infants.

Case 25 Mrs. Ma's history is given above and referred to again because the fetal death in this case, the only one in this group, could be attributed probably to other circumstances, particularly since there was not much pathologic evidence of blood destruction.

Case 27 Mrs. Ma's first baby had severe erythroblastosis and died Apr. 25, 1943, in spite of four transfusions of presumably random (Rh positive) blood. During her puerperium, Mrs. Ma received one transfusion, which was followed by a severe chill and fever. In December 1944 the patient became pregnant again, and tests during her fourth, sixth, and eighth months of her pregnancy gave no evidence of iso-immunization. A specimen drawn one month before delivery contained moderately active blocking antibodies. Accordingly, labor was induced, and the infant was normal at birth (Aug. 6, 1945) but became slightly jaundiced in a few hours. The jaundice deepened gradually for thirty-six hours and then receded slowly, probably because the baby was transfused with Rh negative blood within from two to three hours after delivery. In his letter, Dr. Herrman writes: "I am certain we prevented a more severe case, but it can't be proven."

Of the seven husbands tested, five are Rh negative, so that the fetus in each succeeding pregnancy must be Rh positive. Of the remaining three, one (case 26) must be heterozygous. One of the patients (case 27) had a severe transfusion reaction following her first pregnancy.

Apparently this control group as well as the transfused group selects those Rh negative women who produce antibodies with great ease. The existence of individual differences in response to iso-immunization, probably determined by genetic factors, was postulated by Levine in order to explain the general low incidence of erythroblastosis fetalis, in spite of a high incidence of random incompatible matings.^{3, 17}

It is now believed that erythroblastosis fetalis occurs in about one of 150 to 200 random full term deliveries.^{3, 12, 19} This figure, based on Rh tests to detect all instances of iso-immunization, is to be contrasted with a value of 1 case in 438 deliveries based on clinical grounds only.²⁰ If all Rh negative women responded readily to iso-immunization, one should expect erythroblastosis fetalis to occur in almost all of the 13 per cent of matings in which the father is Rh positive and the mother Rh negative. At the same time, erythroblastosis fetalis in the first full term Rh positive infant should occur very frequently.

Granting that these women were not transfused, one cannot exclude the possibility that the iso-immunization in at least some of them may have been initiated either many years previously or at any time prior to or during a pregnancy by the common practice of administering small amounts of blood intramuscularly.⁴ In the days preceding the use of vitamin K, blood was routinely given to the newborn infant, or to the pregnant woman in repeated doses.* Intramuscular injection of blood is still being used as a form of nonspecific therapy.

* It is of interest that 3 cases of this group were referred by Dr. Herrman, who assured the writer that these three mothers were not transfused prior to their pregnancies. In reply to a query regarding the possibility of intramuscular injection of blood, Dr. Herrman writes: "I'm afraid you will have a hard time getting correct information concerning intramuscular blood in infancy. It has been almost routine with some doctors in the Middle West to give intramuscular blood at the time of delivery, especially if a difficult labor was anticipated. Many of the patients know nothing about it unless their husbands

The objection may be raised that the small quantities of blood used for intramuscular injection may be insufficient to induce iso-immunization. There is, however, considerable support from immunologic literature to indicate that immunization may be stimulated by injection of unbelievably minute amounts of blood or other antigens. So far as iso-immunization by the fetal blood across the placenta is concerned, Levine calculated that the passage of as little as 0.13 cc of fetal blood is required to immunize the Rh negative mother. Indeed, pregnancy and the intramuscular injection of blood provide conditions held to be favorable for iso-immunization, i.e., the continual presence of minute quantities of blood acting over a long period.

By and large, it is not possible to obtain a history of intramuscular administration of blood, but in view of its routine and indiscriminate use in the past, this procedure must be considered as a possible source of the immunization of Rh negative individuals even in infancy or the neonatal period. While infants do not produce antibodies as readily as adults, these intramuscular injections may at least serve to initiate the process. In short, it is advisable to avoid this form of therapy in Rh negative girls or women unless Rh negative blood is used.

Recently, Wiener²² recommended the so-called biologic test to detect Rh incompatibility. * This consists of preliminary intravenous injection of 50 cc of Rh positive blood, and comparison with the naked eye of the color of the patient's original citrated plasma with that of a comparable specimen taken one and one-half hours after the injection. Aside from the fact that the method is cumbersome and not always reliable, it should also be kept in mind that the injection of quantities of much less than 50 cc of Rh positive blood may cause severe hemolytic reactions accompanied by oliguria. In the absence of severe reactions, the injection of small amounts of Rh positive blood may initiate iso-immunization or intensify this process if already induced. If the test is to be used to detect incompatibility due to finer variations of the Rh factor or other blood factors, these considerations are to be kept in mind. The biologic test has very limited value, since the tests *in vitro* (agglutinins, blocking antibodies, slide test of Diamond²⁴) that will detect almost all instances of iso-immunization are less cumbersome, more reliable, and do not inconvenience the patient.

GENERAL CONSIDERATIONS

In the material presented above, two highly contrasting groups of cases were selected in order to determine the role of transfusions prior to pregnancy. However, supporting evidence can be derived from still another group of cases, briefly referred to below.

It is not generally appreciated that the degree of iso-immunization required to induce symptoms of the hemolytic disease in the infant is far more intense than that required to produce a severe hemolytic reaction in the mother following

happen to be the donors. This practice has been dropped in the last few years and vitamin K substituted.²¹ There is no reason to believe that this procedure was limited to any geographic area.

* This test was used previously as a further check on the grouping and compatibility tests.²

transfusion of Rh positive blood. Now that the pathogenesis of erythroblastosis fetalis is established, it is obvious that the hemolytic disease is the result of prolonged intra-uterine action of maternal anti-Rh agglutinins on the susceptible fetal blood. Accordingly, one may expect to find instances of Rh negative women with anti-Rh antibodies who nevertheless have normal Rh positive children,^{3 25 26 27} while in the following pregnancy the degree of iso-immunization is sufficiently severe to produce the hemolytic disease.

In one of the earliest cases studied with Burnham,^{3 25} the blood of an Rh negative mother who had just been delivered of a normal Rh positive infant was found to be incompatible with her husband's group-compatible blood. Because the patient's condition was not serious, the transfusion was not carried out. In 1944, the patient was delivered of an infant that had a mild to moderate form of icterus gravis, with complete recovery. One may speculate that had this patient received and survived the transfusion of Rh positive blood, the pregnancy of 1944 would have ended in fetal death. This indeed was the outcome in two Rh negative women who were transfused with Rh positive blood following delivery of normal Rh positive infants.

Case 28 Patient McC, 30 years old, blood group B, Rh negative, had been married eight years. In 1939, she had a presumably normal child whose blood was of group B, Rh positive, type Rh. After delivery, the mother was transfused with group-compatible blood. Soon after the transfusion was over, the patient had a violent reaction. No other details are available. In 1943, the patient was delivered of a full term fetus that had died twenty-four hours before birth. In 1944, when she was tested for the first time in the fifth month of her third pregnancy, strong blocking antibodies were present and, as was to be anticipated, the end result was fetal death.

Case 30 Mrs. So is in group O, Rh negative. Her first pregnancy was in 1936, and she was delivered of a normal Rh positive infant. (Although not tested, the infant was undoubtedly Rh positive, because the father's blood failed to react with anti-Hr serums. The genotype of the father is most likely Rh_iRh₁.) Shortly after the delivery, the mother received two uneventful transfusions, and it is therefore safe to assume that she was not immunized as a result of the first pregnancy. Each of four subsequent pregnancies (1938 1939 1943 1945) resulted in fetal death. Anti-Rh agglutinins were demonstrable several weeks after the last delivery.

These 2 cases cannot be included in the series because it was the second pregnancy with an Rh positive fetus that presumably resulted in erythroblastosis fetalis. In the first case, the first pregnancy probably immunized the mother, but the antibody production, insufficient to cause the disease in the fetus, was intense enough to induce a severe transfusion reaction. In both cases, the additional antigenic stimulus of transfusions of Rh positive blood probably induced a degree of iso-immunization in the following pregnancies sufficient to induce fetal death. One may well speculate that if these patients had received Rh negative blood, the infant in the second pregnancy might have had a milder form of erythroblastosis fetalis.

DISCUSSION

Soon after the clinical importance of the Rh factor was established, emphasis was placed on the prevention of intragroup hemolytic transfusion reactions by the use of Rh negative donors for all Rh negative patients already immunized either

by previous transfusions or by pregnancies. For an index of iso-immunization, the obstetrician was advised to be guided mainly by the history of fetal and neonatal morbidity. An additional preventive measure that now becomes imperative is the exclusive use of Rh negative blood for all Rh negative patients to be transfused. In other words, the emphasis should now be placed on the *prevention* of iso-immunization. This holds true particularly with regard to the female population of all ages, but the present study was limited to the influence of previous transfusions on the incidence of erythroblastosis fetalis.

The deliberate iso-immunization of the Rh negative female population, even as infants, by transfusion or perhaps even by intramuscular injection of Rh positive blood, can now be prevented. This simple measure should, by itself, reduce the incidence of erythroblastosis fetalis, especially in its more severe forms.^{4, 17} By and large, this is the responsibility to be shared by pediatricians and those hematologists who perform compatibility tests and transfusions.

The fundamental fact is that *once an Rh negative individual is immunized, he or she must be considered as remaining potentially immunized for the remainder of his or her natural lifetime*.⁶ So far as any of our female population is concerned, the outcome of pregnancies, even many years after transfusion, can be influenced by the type of blood used in transfusing Rh negative female infants or girls. Similarly, it is important to determine the Rh factor of an adult female patient before transfusions are carried out. Furthermore, one should not be completely guided by the presence or absence of a history of fetal or neonatal morbidity, particularly if more than one transfusion is contemplated. To be more specific, all Rh negative mothers of normal Rh positive children are liable to serious intragroup transfusion reactions if, even many years later, they receive more than one transfusion.⁶

Although the role of previous intramuscular injection of blood in initiating iso-immunization is not well established, it must be considered as probably significant, since only minute quantities of blood are required to induce iso-immunization. Certainly, with the introduction of vitamin K for the treatment of hemorrhagic disease of the newborn, this factor will become less important.

The more widespread use of Rh tests cannot be recommended without taking into account the matter of a supply of potent anti-Rh serums. It can be assumed that at present there is not yet available an experimental serum of a potency equal to that of human serums. Certainly efforts should be directed in the future toward improvement of the quality of experimental Rh serums.

Undoubtedly there will be sufficient human anti-Rh serums if all women having potent anti-Rh serums are bled periodically. The yield from these women can be increased to a considerable degree by the use of several measures. In the first place, the amount withdrawn should always be replaced by a transfusion of compatible Rh negative blood. Second, the method of Hill and Haberman,²⁸ of maintaining a high degree of iso-immunization in selected cases on a voluntary basis, should be adopted. These workers inject such women intravenously with minute amounts of Rh positive blood after the agglutinin titer of their blood drops below a level sufficient for routine use.

The program, initiated several years ago, of concentrating weak anti-Rh serums,

thus rendering them useful as diagnostic reagents, should be encouraged.²⁹ Furthermore, Levine and Waller³⁰ have shown recently that anti-Rh serums containing both potent agglutinins and blocking antibodies can be absorbed so that the latter antibodies are removed without diminishing to any great extent the agglutinin titer.

The difficulties in obtaining sufficient quantities of potent anti-Rh serums from patients immunized by repeated transfusions are obvious, but occasionally some of the measures suggested above may be applicable.

SUMMARY

The incidence of fatal forms of erythroblastosis fetalis in the first-born can be diminished by the simple measure of transfusing all Rh negative female patients, even as infants, with Rh negative blood. Once a female patient is found to be Rh negative, all subsequent transfusions must be carried out with Rh negative blood. The indications are that sufficient human anti-Rh serums will become available for the more extensive Rh tests required for the prevention of iso-immunization by transfusion.

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RELATION OF CONTACTING SURFACE AND ANTICEPHALIN ACTIVITY TO THE MAINTENANCE OF THE FLUIDITY AND COAGULABILITY OF BLOOD

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TENTATIVE explanations of the mechanism of coagulation of the blood must necessarily be concerned as well with the equally important problem of the maintenance of its fluidity in the circulation. Fluidity and coagulability are properties of the blood that are in a sense antagonistic to each other. To understand what promotes coagulation, one must also be familiar with the factors that discourage this change and thereby keep the blood in a fluid state. Both physical states are essential in their proper places. Excessive bleeding may result when the blood is so stable that it remains liquid longer than it should after it leaves the vessels, if, on the other hand, the blood clots within the vessels, where it should not, an interruption of the circulation may follow. It is by preserving a balance between fluidity-inducing (anticoagulant) and coagulation-promoting (coagulant) factors that the blood maintains itself as a circulating medium.

Two theories of blood coagulation, with substantial experimental support—those of Bordet¹ and of Howell²—have attempted to account for the state of fluidity of circulating blood as well as for the development of coagulation in shed blood. Bordet divided the reactions involved into three phases:

1. Proserozyme (inactive prothrombin) on contact with a suitable surface or with lipid cytozyme = serozyme (active prothrombin)
2. Serozyme (active prothrombin) + cytozyme (lipid from platelets and tissue cells) in the presence of calcium = thrombin
3. Thrombin + fibrinogen = fibrin

Howell also recognized the existence of three successive reactions:

1. Prothrombin / antiprothrombin (heparin) + platelet or tissue factor (cephalin) = free prothrombin
2. Free prothrombin + calcium = thrombin
3. Thrombin + fibrinogen = fibrin

With the exception of Pickering³ and Fuchs,⁴ most workers, especially in publications within the last ten years, do not mention the first phase and therefore leave unexplained the question of the maintenance of the fluidity of circulating blood.

In common with Bordet, Howell recognized that the prothrombin in circulating blood differs from that in shed blood. According to Howell, the circulating prothrombin is bound with antiprothrombin (heparin) and is freed from the latter by the platelet or tissue factor that neutralizes the antiprothrombin. With this point Bordet could not bring himself to agree, and much of the evidence that he brought against it received further support from Pickering,³ Mellanby,⁵ and others. Bor-

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det¹ also questioned the hypothesis that prothrombin can be activated by calcium alone, and insisted that the lipid (cytozyme) activator is necessary

According to Bordet's theory, the initial step toward clotting is a change of the inactive prothrombin (proserozyme) into an active one (serozyme) when the blood is brought into contact with a suitable surface or with the lipid from platelet and tissue cells (cytozyme). Just how contact brings about this change, which requires a variable period of time, was not clear to Bordet. He recognized, however, that the inactive form of prothrombin (proserozyme) may simply represent prothrombin protected by an inhibiting substance that can be inactivated or displaced by a suitable contacting surface, thereby setting the prothrombin free.¹

The existence of forces that influence the conversion rate of prothrombin was therefore clearly recognized by Bordet and Howell. Within recent years, principally perhaps because of the great interest in the quantitative determination of prothrombin, the role of the contacting surface and the existence of prothrombins of differing reactivity have been to some extent overlooked. The process of coagulation has generally been divided into the two phases originally proposed by Mcra-witz⁶

1. Thrombokinas (or thromboplastin) + prothrombin + calcium = thrombin

2. Thrombin + fibrinogen = fibrin

The impression is conveyed (unwittingly perhaps) that prothrombin can be instantaneously changed into thrombin *in vivo* or *in vitro* by the action of thromboplastin in the amount ordinarily present in the blood. The fact that a great excess of thromboplastin is necessary for the rapid conversion of prothrombin to thrombin in the quantitative determination of prothrombin, should not lead one to forget that such working conditions are artificial and designed to remove natural obstacles (presence of inhibitors, variations of prothrombin conversion rate) that have in the past defeated efforts toward the development of quantitative methods. Attention has been drawn to the errors that may be caused by this oversight.⁷ The tendency to attribute to the amount of prothrombin in the blood the dominating role in coagulation overlooks the fact that, under natural conditions, the conversion rate of prothrombin (independent of its amount) really governs the speed of the reaction. For example, what seem to be quantitative differences in the prothrombin of the blood of different species have been shown⁸ to represent variations in the respective prothrombin conversion rates. It becomes of some importance therefore to determine the factors involved in the conversion of prothrombin to thrombin.

The remarks that follow will deal principally with the *natural* forces involved in the changes preceding the activation of prothrombin in *man*. There is general agreement that the lipid from platelets and tissue extracts promotes the coagulation of blood by accelerating the transformation of prothrombin into thrombin.^{9,10,7} If the amount of prothrombin is held fixed, the speed of its conversion into thrombin is substantially determined by the quantity of lipid factor available. Whether the lipid influences the conversion of prothrombin by combining directly with it, as Bordet,¹ Mellanby,⁵ and others have maintained, or by removing an inhibitor,² is a point on which general agreement has not been reached. The evidence brought out by modern methods seems to indicate that thrombin results from

a chemical combination between the lipid factor and prothrombin. The question that remains unanswered is: What factor keeps the prothrombin relatively invulnerable to the amounts of the thromboplastic lipid that may be released in the circulating blood, and how is that factor removed from the blood as it is shed?

The existence in the blood of a natural antiprothrombin, or of a substance that acts on prothrombin itself and presumably opposes its transformation into thrombin, is not generally conceded.¹⁰ Natural antithromboplastins likewise have until recently¹¹ not been seriously considered. These doubts have been reflected in the attitude of some workers,^{12, 13} who have been unwilling to accept the assumption of an inhibitor as an explanation of the fluidity of circulating blood. This viewpoint is all the more understandable when one considers that the inhibitor, if present at all, would make itself felt only in the early stages of coagulation, before adverse surrounding conditions or release of the lipid, platelet, or tissue thromboplastic factor, rendered it partially or wholly ineffective. It would therefore have a fleeting existence *in vitro*. The difficulty of distinguishing between antiprothrombin and antithromboplastic activity contributes further to the uncertainty. Since, for activating prothrombin, thromboplastin is required, the slowing of this activation might be attributed with equal plausibility to a deficiency in thromboplastin, to antiprothrombin, or to antithromboplastic activity. In view of what follows, it is not unlikely that much that has been described as antiprothrombin may actually be antithromboplastic activity.

Observations of the past four years seem to indicate that a natural antithromboplastin exists in normal human plasma.¹⁴ This substance (or group of substances), the activity of which may be detected in plasma separated from normal blood collected with special precautions,¹⁴ reduces the clot-accelerating action of dilute extracts of brain tissue when the plasma is incubated with the extracts before recalcification. The inhibiting activity is exhaustible, has a certain degree of species specificity, is made ineffective by heating (to 65°C. for five minutes) or dilution, diminishes slowly on standing in paraffin- or collodion-coated tubes, and more rapidly in glass vessels when in contact with platelets or other blood and tissue cells. Hemophilic plasma has an inhibiting activity from five to eight times greater than that of normal plasma in relation to certain dilute thromboplastin solutions. In shed hemophilic blood, more free thromboplastin and a longer time are necessary for the inactivation of antithromboplastin than in normal blood.¹⁴

The thromboplastin of aqueous brain extracts is a lipoprotein, its clot-accelerating properties reside chiefly in the lipid moiety, the active constituent of which is a cephalin.² It seems that it is the cephalin* portion that is vulnerable to the antithromboplastic activity of the plasma.¹⁵ The well known increased resistance of hemophilic plasma to activation by cephalin appears to be due to its excessive antithromboplastic activity. This activity, which may then be properly referred

* The term cephalin as used in this and preceding papers designates the alcohol insoluble lipid fraction extracted with ethyl ether from acetone-dried human brain. Cephalin suspensions so obtained are obviously not pure.¹ Most indications point to a cephalin as the active fraction of the thromboplastic lipoprotein¹⁰ and it may be so considered until convincing evidence to the contrary is available. The term anticephalin is intended to designate that activity of plasma which is directed against the thromboplastic action of cephalin.

to as due to *anticephalin*, bears some resemblance to the anticoagulating effect of the protamines,¹⁵ which are known to oppose the thromboplastic action of cephalin by combining with it and forming indissociable inert compounds¹⁶

Plasmas that have been exposed to contact with materials rich in silica (kaolin, asbestos wool fibers, infusorial earth, glass), or that have been dialyzed against water, lose their anticephalin activity and in this state are easily activated by cephalin, in the form either of tissue or platelet extracts or of cephalin suspensions. A study of the rate of conversion of prothrombin of these plasmas (hemophilic, normal, and adsorbed) has disclosed that the prothrombin of the adsorbed plasma is more rapidly converted into thrombin than that of the normal and hemophilic plasmas.¹⁸ Dilutions of the plasma to 5 per cent or less may be required to equalize the reaction to cephalin as between normal and hemophilic plasmas.^{18, 19} On the other hand, in order to equalize the coagulability of adsorbed plasma, normal plasma requires 320 times and hemophilic plasma about 1000 times as much cephalin as adsorbed plasma (table 1).

TABLE 1—Clotting Time* (in Seconds), in Glass Tubes, of Normal, Hemophilic, and Adsorbed Plasmas to Which Increasing Dilutions (0.85 per cent Sodium Chloride) of Cephalin Have Been Added

Type of Plasma	Dilution of Cephalin Suspension										0.85 % NaCl
	0	× 5	× 10	× 20	× 40	× 80	× 160	× 320	× 500	× 1000	
Normal	80	89	101	110	118	127	147	168	198	218	265
Hemophilic	169	258	304	380	543	1168	1522	>1800	>1800	>1800	>1800
Adsorbed†	27	29	34	40	52	59	67	80	110	173	210

* 0.3 cc. of plasma, 0.1 cc. of cephalin suspension, 0.1 cc. of 0.074 mol calcium chloride

† Hemophilic plasma in contact with asbestos wool fibers for two hours at 20 C. (10 mg. of asbestos per 1 ml. of plasma)

Prothrombin content (two stage method) of normal and hemophilic plasma, 100 per cent of normal, of adsorbed plasma, 78 per cent of normal

The fact that contact with certain surfaces accelerates blood coagulability seems therefore to be linked with the removal (by adsorption or inactivation) of anticephalin from the plasma, with a consequent increase in its prothrombin conversion rate. Plasmas with an excess of anticephalin (e.g., hemophilic) appear to be better protected not only from activation by cephalin but also from the effect of contact with glass.¹⁹ The slower rate of coagulation in paraffin- and collodion-coated tubes seems to be due to the fact that they preserve anticephalin activity longer, thereby holding the conversion of prothrombin to a slow rate. Differences between the coagulability of blood in glass and in collodion tubes generally parallel the magnitude of its anticephalin activity. The high direct correlation between these two variables led to the proposal that the extent of the difference between the rate of coagulation of blood in glass and in collodion tubes might be used as an approximate estimate of blood anticephalin activity.²⁰ The greater magnitude of the electrical charge on a glass surface in contact with water, when compared with that on a paraffin surface, led Gortner and Briggs^{20a} to suggest that a positively charged substance in blood standing in a glass tube, may be adsorbed by the negatively charged

glass surface, thereby modifying the coagulability of the blood. That such forces may be involved in changes in the plasma after contact with glass, has been indicated by Lozner and Taylor.¹⁰ Whether anticephalin is simply adsorbed on the contacting surface or also inactivated is not possible to state at present, efforts at elution from the adsorbents have, so far, yielded inconclusive results.

Potent suspensions of cephalin may be injected intravenously without visible change in the animal, except for a transient blood hypercoagulability.^{11, 12} Circulating blood seems to adapt itself to the injection of thromboplastic substances intravenously, provided the mechanism of adjustment is not overwhelmed by too rapid injection of concentrated solutions. From the foregoing it seems justifiable, therefore, that anticephalin should be included among the various factors contributing to the maintenance of the fluidity of blood.

What relation does this activity have to heparin, the anticoagulant extracted from tissues? It is generally agreed that, with the aid of plasma cofactors, heparin acts as an antithrombin and in addition is capable of retarding or preventing the transformation of prothrombin into thrombin, an effect that could with equal plausibility be considered antithromboplastic or antiprothrombic.^{23, 24, 5} That the anticephalin activity of the plasma, in itself, is not due to heparin alone and cannot be replaced by it, is indicated by the fact that once anticephalin is removed from plasma by contact with adsorbents, or by dialysis (with restoration of salt), heparin can no longer block the conversion of the prothrombin of such plasma.²⁵ Moreover, as Chargaff points out,^{26a} heparin cannot neutralize cephalin, as both substances are strongly acidic. Since, in plasma brought in contact with adsorbents, anticephalin activity may be removed while prothrombin is preserved—though addition of heparin before or after contact will not retard prothrombin conversion in such adsorbed plasmas—the designation of heparin as an antiprothrombin seems open to question. Furthermore, hemophilic blood with a known increase in anticephalin activity does not contain, according to Howell,^{25, 26} an excess of heparin. Experiments under way²⁷ seem to indicate that heparin blocks the conversion of prothrombin to thrombin by intensifying or enhancing the natural anticephalin activity of the plasma. These considerations make it seem desirable, for the time being, not to regard the natural anticephalin activity of the plasma as a manifestation of the presence of heparin.

With the foregoing in mind, let us see how anticephalin activity and its so far known characteristics may be fitted into an explanation of the mechanism for maintenance of the fluidity and coagulability of blood. Circulating blood seems to have within and about itself all that is required to delay or promote coagulation. The stability of the blood depends on the extent of dominance of one of the following opposing groups of factors over the other.

Anticoagulants (Fluidity promoting agents)	Coagulants (Coagulation promoting agents)
1. Intact vascular endothelium (or a surface like collodion)	1. Damaged vascular endothelium (or a surface like clay or glass)
2. Anticephalin (antithromboplastic)	2. Cephalin (platelets, leukocytes, tissue juices)
3. Antithrombin	3. Prothrombin Ca^{++10}
4. Fibrinolysin	4. Fibrinogen

It should follow from the foregoing that the coagulability of a given sample of blood may be enhanced by an increase in the effective amount of the coagulation-promoting factors or by a decrease in the fluidity-promoting elements. Conversely, blood may become unusually stable if there is an *uncompensated* increase in the anti-coagulants or a decrease in the coagulants.

THE CONTACTING SURFACE

The internal area of a blood vessel is lined with endothelium, which offers to the blood a neutral contacting surface—neutral in the sense that it does not readily precipitate the changes that precede the inception of clotting. In this respect the surface endothelium of the vessel resembles a collodion membrane. As long as the stability of the blood is maintained by an adequate content of clot-inhibiting substances, surfaces like collodion or the endothelial lining of blood vessels will aid in maintaining this stability. When a vessel is severed, the entrance of tissue juices into the circulation at the point of contact in the ruptured area, and the destruction of the continuity of the endothelium, will interfere with the fluidity of the blood (*a*) by supplying thromboplastin, which inactivates the available anticephalin and thus accelerates the conversion of prothrombin, and (*b*) by offering a surface favorable to the removal of anticephalin and the liberation of more cephalin from disintegrating platelets. Blood itself may, on the other hand, even without previous vascular injury, lose its anticephalin activity and consequent stability, for one reason or another (stasis, hemorrhage). A neutral undamaged endothelial surface will then afford it little protection and intravascular clotting may conceivably take place, even though the vessel surface remains intact.

DEGREES OF STABILITY OF THE BLOOD

The stability of blood is therefore influenced by the surface with which it comes into contact and the extent of the blood anticephalin activity, among other factors. Table 2 presents examples of blood with varying degrees of stability. Hemophilic blood resists contact with glass and activation by cephalin, and displays more anticephalin activity than normal blood. On the other hand, normal blood has greater stability than blood obtained after a severe hemorrhage. The latter is more readily activated by cephalin, has less anticephalin activity, and is not greatly influenced by differences in contacting surfaces.

It seems probable that blood of low stability with respect to coagulation may lose its fluidity readily in the circulation, even when in contact with surfaces like the endothelium of blood vessels, which probably contribute to the stability of blood by preserving anticephalin activity. On the other hand, blood like that of the hemophilic will maintain its stability, even though the vessel wall has been traumatized and the character of the endothelial surface rendered favorable to coagulation. The excess of anticephalin in this blood will protect it against these surface conditions, just as it protects shed blood of this kind against glass surfaces.

These remarks have been intended principally to cover the factors involved in the first phase of coagulation in their relation to the maintenance of the stability of blood. It is obvious that increased stability can also result, for example, from a marked increase in antithrombin (rarely encountered in man) or from a diminution

in the prothrombin of blood while its anticephalin activity is maintained, an absence of fibrinogen would produce a similar effect. It must be kept in mind, however, that a simple diminution in prothrombin may not in itself increase the stability of the plasma if there is an equal or greater decrease in anticephalin activity. Increased fluidity or coagulability must, in the end, result from an *uncompensated* increase or decrease in the anticoagulant or coagulant factors.

There have been observations^{27, 22} of patients in whose plasma a decrease in the amount of prothrombin has apparently been compensated by an increase in its convertibility. In such patients the rate of coagulation of blood may remain unaltered and may even sometimes become accelerated. After severe hemorrhage, although the amount of prothrombin may not change or may even be reduced,

TABLE 2.—*Coagulation Time of Venous Blood in Glass and Lusteroid Tubes after Addition of Cephalin and of Plasma before and after Incubation with Cephalin*

	Normal*		Hemophilic		Posthemorrhagic†	
	Glass	Lusteroid	Glass	Lusteroid	Glass	Lusteroid
Blood‡ cephalin clotting time (sec.)	124	244	830	1449	86	112
Plasma§ cephalin clotting time (sec.)						
0' incubation	91	194	238	618	71	96
20' incubation	114	362	345	2296	76	64
Prothrombin (percentage)						
one stage method	100		100		> 100	
two stage method	100		118		96	

* Means of 26 determinations in 19 men.²⁰

† Patient with bleeding gastric ulcer.

‡ 1 cc of blood + 0.1 cc of cephalin suspension at 38 C.

§ 0.3 cc of citrated plasma, 0.1 cc of cephalin (0' or 20' incubation), 0.1 cc of 0.074 mol calcium chloride, in 13 mm i.d. tubes at 38 C.

there is often a diminution in anticephalin activity,²⁸ with a consequent acceleration of the prothrombin conversion rate and of the blood clotting time.

Slight or moderate increases in stability of the blood, on the other hand, occur spontaneously in man, but are generally undetected, principally because the rate of coagulation of whole blood is usually measured in tubes of glass, a surface least suited to study of such changes. The blood of 2 patients with histories of bleeding that suggested hemophilia, yielded clotting times in glass tubes not significantly greater than normal (table 3). The cephalin and whole blood clotting times in plastic tubes revealed the true nature of their disorder. Similar discordant results as regards the coagulability of hemophilic blood, with or without addition of cephalin, are often found after a transfusion of normal blood. There may be a significant shortening of the clotting time in glass tubes, while little or no change will be noted in paraffin or plastic tubes. Attention has been drawn by Davidson and

McDonald²⁹ to the advantages of plastic tubes for detection of slight changes in blood coagulability induced by dicumarol

Plasma separated (high speed centrifuging in cold) from normal or hemophilic blood within five minutes after collection with special precautions and without use of anticoagulants, displays unusual stability when kept in paraffin- or collodion-coated tubes³⁰ but clots promptly when placed in contact with glass. If

TABLE 3—*Comparison of Coagulation Time of Venous Blood and Plasma of 2 Mildly Hemophilic Subjects with That in Normal Men*

	Normal		Hemophilic			
	Glass	Lusteroid	Subject 1		Subject 2	
			Glass	Lusteroid	Glass	Lusteroid
Blood						
Clotting time (sec.) ± S D	615* ±80	1953* ±374	720	3480	960	3120
Cephalin clotting time (sec.) ± S D	124* ±13	244* ±38	285	617	371	730
Plasma						
Cephalin clotting time (sec.) 0' incubation ± S D	91† ±13	194† ±41	122	341	193	415
20' incubation ± S D	114† ±15	362† ±109	165	574	221	683

* Means of 26 determinations in 19 normal men

† Means of 33 determinations in 26 normal men

Prothrombin content of blood of hemophilic subjects 100 per cent of normal

TABLE 4—*Clotting Time in Glass and Collodion tubes, of Mixtures of Hemophilic and Normal Plasma in Various Proportions (0.3 cc of Plasma, 0.1 cc of Calcium Chloride)*

Plasma									
normal (cc)	0.3	0.27	0.25	0.2	0.15	0.1	0.05	0.03	0
hemophilic (cc)	0	0.03	0.05	0.1	0.15	0.2	0.25	0.27	0.3
Clotting time (sec.)									
glass tube	232	235	242	275	290	300	355	370	3120
collodion tube	610	687	860	932	1077	1145	1265	2880	>7200

the effect of addition of hemophilic blood or plasma to normal blood or plasma is tested in glass tubes, there is a striking reduction of the clotting time toward normal. In collodion tubes, however, the results differ. A clot-delaying effect may be detected after the addition of one part of hemophilic to nine parts of normal plasma (table 4). This effect may be decreased or even erased by dilution, heating, or exposure of the hemophilic plasma to glass or certain adsorbents. From the data (table 4) one might with just as much propriety maintain that the coagulability

of normal plasma is decreased by the hemophilic plasma as that the clotting time of the latter is accelerated by the normal plasma. These difficulties of interpretation are intrinsic to tests of this type. The apparent clot-accelerating effect of normal plasma, however, becomes even less convincing when the mixtures of the normal and hemophilic blood or plasma are made at once after collection, in tubes of plastic, paraffin, or similar composition.

Some of the earlier workers on blood coagulation were able to retain, either through choice or necessity, almost completely natural conditions in the planning and execution of their experiments. It was principally on the basis of observations on the behavior of blood in intra- and extracorporeal segments of vessels and in glass tubes that Lister³¹ was able to state

The blood as it exists within the vessels has no spontaneous tendency to coagulate and therefore the notion of any action on the part of the blood vessels to prevent coagulation is entirely out of the question. The peculiarity of the living vessels consists not in any such action upon the blood but in the circumstance remarkable indeed as it is, that their living membrane when in a state of health is entirely negative in its relation to coagulation and fails to cause that molecular disturbance or if we may so speak, catalytic action which is produced upon the blood by all ordinary matter.

The real cause of the coagulation of the blood when shed from the body is the influence exerted upon it by ordinary matter the contact of which for a very brief period effects a change in the blood inducing a mutual reaction between its solid and fluid constituents in which the corpuscles impart to the liquor sanguinis a disposition to coagulate.

SUMMARY

A review of the various factors in the blood that have to do with the promotion and the retardation of coagulation is presented.

Circulating blood seems to have within and about itself all the factors required to delay or to promote coagulation. The stability of blood (i.e., its tendency to remain fluid) depends on the extent of the dominance of the anticoagulant (fluidity-inducing) group of factors over the coagulant (coagulation-promoting) group.

Among the anticoagulant factors are the natural anticephalin activity of the plasma and the intact vascular endothelium, the latter is simulated by such contacting surfaces as collodion and paraffin films.

Alterations in the stability of blood result from *uncompensated* increases or decreases in one or more of the anticoagulant or coagulant factors.

The increased stability of hemophilic blood, due to an uncompensated excess of anticephalin activity, enables it to resist activation by cephalin or by contact with injured walls of blood vessels or with surfaces like glass. Blood obtained from normal individuals after severe hemorrhage has a decreased stability owing to an uncompensated diminution in anticephalin activity, such blood is readily clotted by cephalin and may not remain stable even when in contact with undamaged vascular endothelium or surfaces like collodion or paraffin.

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THE RELATION OF CERTAIN FRACTIONS OF THE PLASMA GLOBULINS TO THE COAGULATION DEFECT IN HEMOPHILIA

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A SERIES of reports from the Thorndike Memorial Laboratory^{1 2 3 4 5 6} have indicated that normal cell-free human plasma contains some factors which are deficient in hemophilia. There is evidence that by the parenteral administration of derivatives of normal cell-free plasma in hemophilia, the coagulation defect can be modified toward normal. The present communication reviews some of the older work on this subject and presents new evidence accumulated during the past three years.

One of the earliest studies on the use of fresh whole blood in the treatment of hemophilia was made by Minot and Lee in 1916.⁷ They ascribed the beneficial effects of transfusion of whole blood to the platelets thus added to the patient's circulating blood. In the same year, Addis⁸ showed that the administration of whole blood or of serum reduced the coagulation time of hemophilic blood. Patek and Stetson¹ reinvestigated this problem in 1935 and found that small transfusions, as little as 30 cc., of fresh whole blood were as effective as large amounts in the initial reduction of the coagulation time in hemophilia, but that the reduction of the coagulation time was much more transient when these small amounts were used. These authors found that the beneficial effects of fresh whole blood could be duplicated by the use of fresh normal human plasma. They then deprived the plasma of its platelet content and found that the filtrates so obtained were equally effective in reducing the coagulation time of hemophilic blood *in vivo*. From the foregoing evidence it seemed reasonable to conclude that fresh normal whole blood or platelet-free plasma contained a material which was effective in shortening the coagulation time of hemophilic blood. Transfusions of hemophilic blood or of hemophilic plasma failed to have such a beneficial effect.⁹ In other words, there seemed to be evidence that hemophilic blood was deficient in some factor or factors present in normal blood.

Competent investigators have shown that the coagulation defect in hemophilic blood could not be related to deficiency in calcium ion,^{10 11 12 13} prothrom-

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The products of plasma fractionation used in this work were developed from blood collected for the American Red Cross by the Department of Physical Chemistry, Harvard Medical School, under contract recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Harvard University.

The nomenclature concerning plasma fractions used in this publication is that reported by Cohn and his associates.^{22 24}

bin^{14 15 16 17} or fibrinogen^{18 19 8 11 12} of the plasma. Consequently, our investigations were directed toward an attempt to determine where the deficiency, if any, resided. It was determined that certain globulin fractions prepared by acid precipitates of diluted cell-free plasma at pH 5.5 and also similar globulin preparations obtained by simple dialysis against distilled or tap water contained all the antihemophilic properties of the parent plasma.² Similar preparations from hemophilic plasma had little or no coagulation properties.² The acid-precipitable globulins, unfortunately, produced a refractory phase during which second, third and subsequent injections given within six hours were inactive. This method of preparation was therefore discarded.⁴ Euglobin fractions obtained by dialysis could be repeatedly administered without the development of the refractory period.³ Similar preparations of antihemophilic globulins were prepared independently by Bendien and Van Creveld.²⁰ Later these observations were confirmed by Howell.²

It would appear that the antihemophilic properties of these globulin preparations were not due to the removal of inhibitory materials, since the parent plasmas were active and the supernatant fluids from the globulin precipitants had no inhibitory activity and only minimal coagulation activity. Furthermore, hemophilic plasma treated by the same method did not acquire any increase in antihemophilic properties.

Protein fractions prepared as we have just indicated contain both prothrombin and fibrinogen. Since the administration of excessive amounts of these materials might possibly have an effect in reducing the coagulation time, plasmas relatively free of prothrombin and of fibrinogen were prepared by heating normal cell-free plasma to 56°C for two minutes and subsequently passing the filtrate five times through a Seitz filter. Such plasma preparations retain their antihemophilic properties unimpaired.⁶ Euglobin preparations of such plasmas, while not as potent as the parent plasmas, retain a considerable amount of their power to reduce the clotting time of hemophilic blood *in vitro* and *in vivo*.²²

The exact nature of the antihemophilic material is not known. It is definitely associated with the plasma globulins, but whether or not it is a lipoprotein has yet to be determined. Howell² termed the material plasma thromboplastin, but evidence is lacking as to whether or not this nomenclature is acceptable. Studies concerning this matter are at present under way.

As part of the blood substitutes program conducted during the war, large quantities of human plasma have been subjected to multiple fractionation by Dr. E. J. Cohn and the workers in the Department of Physical Chemistry at the Harvard Medical School. Various subfractions of the globulin group have been extensively tested for antihemophilic activity in the Thorndike Memorial Laboratory.²³ The antihemophilic activity was found to be largely concentrated in fraction I and subfraction II of fraction III of the plasma proteins.* A small amount was found in fraction IV. Fraction I contains above 60 per cent of fibrinogen, with small amounts of the other globulins. Subfraction II of fraction III contains 75 per cent

* Nomenclature of Cohn and associates.²⁴

beta globulin and most of the prothrombin Fraction IV contains 55 per cent alpha globulin and 28 per cent beta globulin ²⁴

Extensive studies on the antihemophilic properties of fraction I are being carried out on adult patients in the Boston City Hospital. So far it has been found that single intravenous doses of 200 to 600 mg. of fraction I in volumes of 5 to 10 cc. of isotonic solution of sodium chloride shorten the coagulation time of hemophilic blood as much as do therapeutic doses of whole blood or plasma,³ which reduce the clotting time of such blood to normal. Intramuscular administration of the fraction has been found to give inconstant results and frequently causes painful hematomas.²⁵ Repeated intravenous doses of fraction I are not followed by a refractory period.²⁶ As has been stated, fraction I contains at least 60 per cent of fibrinogen. If it is possible to subfractionate it further in such a manner as to retain its antihemophilic properties while removing the fibrinogen, perhaps even more potent antihemophilic material can be made. It has been found²⁶ that the removal of fibrinogen from fraction I by simple solution and heating to 56 C. for two minutes does not destroy the antihemophilic properties of the fraction, although subsequent tests with thrombin show that no fibrinogen remains in the preparation. Thus, it may be possible to obtain preparations sufficiently potent to make small daily injections feasible, and in this way to maintain the clotting time of hemophilic patients within normal limits.

A *pool of hemophilic plasma* is now being fractionated by the Harvard Pilot Plant.* So far only fraction I of this plasma has been tested. Although the total nitrogen and fibrinogen content of the fraction are within normal limits, no antihemophilic activity has been found in vitro. Sufficient hemophilic fraction I was obtained to make one test in vivo, and in the subject used little or no clot-promoting ability was detected. Details of these observations will be published elsewhere. In the evidence at hand at present there seems to be considerable confirmation of the theory that hemophilic blood is deficient in a factor or factors which are closely associated in chemical fractionation with prothrombin and fibrinogen and which may be separated with some losses from both of these proteins.

Normal human cell-free plasmas when treated with chloroform develop strong proteolytic activity.²⁷⁻³⁴ It has been shown that a true protease is obtained with an optimum pH between 7 and 8 and that the enzyme is capable of digesting fibrinogen, fibrin, gelatin and casein. The enzyme can also apparently produce thrombin from prothrombin.^{32, 34} In relation to blood coagulation, small amounts of the enzyme produce coagulation, larger amounts produce coagulation with subsequent fibrinolysis and, in still higher concentrations, fibrinogenolysis.^{33, 34}

On the other hand, hemophilic plasma as compared with normal plasma develops little proteolytic activity following treatment with chloroform.²⁵

Globulin preparations possessing antihemophilic activity likewise can act as progenitors of such an enzyme system as has been described. Globulin preparations made by simple dialysis do not as a rule have proteolytic activity prior to treat-

* Courtesy of Drs. J. T. Edsall, L. E. Strong and S. M. Armstrong, with whom the complete observations will be published elsewhere.

ment with chloroform. However, fraction I and subfraction II of fraction III of Cohn have on occasion shown preformed lytic activity.⁷³

Upon treatment with chloroform fraction I, subfraction II of fraction III and fraction IV all develop proteolytic activity capable of digesting both fibrin and fibrinogen as well as gelatin and casein. The distribution of the activity between the fractions varies, but it is markedly reduced in subfraction IV of fraction IV and is greatest in subfraction II of fraction III, which likewise contains free enzyme.⁷³ The parent plasmas have been better sources of enzymes after chloroform treatment than have any of the individual fractions of the globulins. However, when the yield of enzyme of all the fractions is considered collectively, as measured by production of nonprotein nitrogen from casein, there is a definite increase in the nitrogen produced per milligram of original protein.⁷³ This may be due to the removal of inhibitor substances or of competitive substrates by the fractionation process. It has already been determined in a few experiments that plasma albumin,* which is not itself digested by the protease, markedly lowers the nonprotein nitrogen produced from gelatin by the plasma protease.⁷⁶ Fraction IV of Cohn has been found to contain both enzyme progenitors and also inhibitor materials.⁷³

The foregoing facts suggest that normal human cell-free plasma contains the progenitor of an enzyme system, which may be elaborated by treatment with chloroform. In hemophilic plasma this enzyme system may be present in reduced amounts. The facts also suggest that the enzyme system plays an as yet unknown role in the process of the coagulation of blood. Further fractionation of the plasma globulins and further studies on the characteristics of the system are necessary to determine the true physiologic action of the enzyme.

Of equal importance with the shortening of the coagulation time of the circulating blood in hemophilia is the local control of hemorrhage when this occurs. The antihemophilic globulin substance discussed earlier in this paper acts as a local hemostatic when applied in dry form with adequate dressings to the bleeding point.³⁷ For some time preparations of this globulin substance from bovine plasma were used in this clinic. Local hemorrhages may be arrested by such preparations in a few minutes. However, antihemophilic globulin preparations act apparently through the normal coagulation reaction and depend for their activity on the release of thrombin from prothrombin, hence instantaneous arrest of hemorrhage does not occur.

Another globulin preparation may be obtained from human, bovine, rabbit or swine plasma³⁸ by a salting-out procedure. Parfentjev^{39, 40} first described such a preparation from rabbit plasma. Subsequent investigators found that these pseudoglobulin preparations were thrombic in nature⁴¹, that is, they acted directly on fibrinogen without the intervention of calcium ion or prothrombin. Since no thromboplastin was added in the preparation, it must be concluded that the salting-out process caused the spontaneous conversion of prothrombin to thrombin. Such spontaneous conversions have been described by Milstone.⁴²

* Provided by Dr J. T. Edsall

From the practical point of view this hemostatic globulin is definitely superior to the antihemophilic globulin in the control of hemorrhage, since its action is immediate. In persons with hemophilia it has been used to control bleeding following amputations, debridements and dental extractions. In normal persons also it has been widely used, a notable example being its employment as a physiologic glue in skin grafting.^{43, 44}

At the present time much fractionation of human plasma proteins is being carried on and thrombin of human origin is available. However, the fact that hemostatic globulin can be obtained not only from human but also from bovine, rabbit and swine plasma may be of great economic importance in the postwar period, when human plasma products will probably not be as available as they are at present.

The question of possible toxic reactions to animal globulin preparations applied locally immediately arises. The last word on this has not yet been written. It may be reported that using a rabbit hemostatic globulin* we have noted no untoward local effects or systemic reactions in many multiple local applications of the substance in the control of hemorrhage in hemophilic and normal subjects.

From the academic point of view, it is of interest to note that while active antihemophilic globulin preparations give rise to a proteolytic enzyme system, either spontaneously or upon treatment with chloroform, hemostatic globulin preparations are devoid of this property. This has been found true not only of a thrombic preparation made by Parfentjev's method but also of thrombin prepared by conversion of prothrombin by the addition of thromboplastin as described by Astrup and Darling⁴⁵ and Seegers.⁴⁶ Thrombin preparations from human plasma prepared by the technic used in the plasma fractionation plant at the Harvard Medical School are likewise devoid of this property, moreover, they do not spontaneously lyse fibrin clots.

CONCLUSION

Hemophilic blood appears to be deficient in some activity associated with the globulin fraction of the plasma protein. The chemical identity of the missing factor or factors is not at present known. For want of a precise name for this factor, we have called it globulin substance, and in this report it has been termed antihemophilic globulin, which term will probably be used in studies now being undertaken by other investigators. However, it should be remembered that until final proof is obtained both of these terms imply an association rather than an identification of the antihemophilic activity with the plasma globulins.

Normal cell-free plasma and the antihemophilic globulin preparations derived therefrom will on treatment with chloroform give rise to a proteolytic enzyme system having some as yet undefined role in blood coagulation. The globulin preparation, but not the original plasma, except under conditions described elsewhere,⁴⁷ will spontaneously produce such an enzyme system. In fractionations of human globulins such spontaneous production of lytic agents has been found in subfraction II of fraction III, and occasionally in fraction I.

* Hemostatic globulin donated by the Lederle Laboratories

Hemophilic plasma does not produce such proteolytic enzyme systems in optimal amounts. Whether this is due to the lowered amount of the precursor (possibly antihemophilic globulin) or to the presence of inhibitor materials is at present unknown.

The intravenous injection of antihemophilic globulin results in a marked acceleration of the clotting time of hemophilic blood. So far only fraction I of the plasma globulin has been used clinically. The dose of the material has not yet been determined, but single injections of 200 to 400 mg. of the material will keep the blood of a hemophilic patient at low coagulation levels for from eight to twelve hours. Repeated injections may be used without the development of a refractory phase.

For local hemostasis a pseudoglobulin may be prepared from human, bovine, swine or rabbit plasma by a salting-out procedure. In this clinic thrombin from rabbit plasma has been used without untoward local or systemic reactions in amputations, debridements and dental extractions in hemophilic patients.

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EDITORIAL

AT first glance, the spleen would seem to be a rather useless organ. Its removal in the normal human being or animal is unattended with any noticeable clinical effects. Careful laboratory examinations under such circumstances reveal, however, that the spleen and the blood are closely related. The red cells become thinner and show Howell-Jolly bodies, the leukocytes and the platelets become greatly increased, and the output of urobilinogen in the feces becomes definitely diminished.¹ From these observations it would appear that the spleen normally makes red cells thicker, has effects on the denudation of normoblasts in the marrow, and in some measure controls the growth and/or delivery of granulocytes from the marrow to the blood. The old adage that the spleen is the graveyard of the red cell cannot now pass unchallenged,² even though the output of bile pigment in the feces is definitely diminished after splenectomy. How the red cell normally becomes broken down still remains something of a mystery. The rather glib explanation that the reticulo-endothelial system destroys it fails to measure up to careful histologic studies, which show no hint of phagocytosis. Other explanations which invoke a rather mysterious lysolecithin, the high lecithin content of the thoracic duct, etc., likewise fail to satisfy. Perhaps the red cell after its many thousand passages through the miles of capillaries and sinusoids, gradually wears out, and becomes hemolyzed in the circulation. The spleen, by tending to make it thicker (i.e., spherocytic), probably helps to wear it out. This purely mechanical function of the spleen is far less intriguing than possible hormonal effects of that organ upon the bone marrow. These are concerned, not only with the red cells, but with the leukocytes and platelets as well.

Thus far, very little direct evidence for the normal existence of splenic hormones has been adduced, although in a recent article, Ungar³ reports the isolation from the spleen of guinea-pigs of a material in crystalline form which he calls splenin. This material was found to reduce the bleeding time, increase the capillary resistance, and inhibit the release of histamine from blood cells. The spleen, as an endocrine organ, is thought by Ungar to play a role in the control of protein metabolism and in certain reactions to stress. These observations require confirmation and amplification, e.g., effects on platelets were apparently not studied.

Normally, the splenic physiology is probably of minor importance, although it is concerned to some extent in the maturation and release of the various cells from the marrow to the blood. This implies the presence of one or several splenic hormones. Under certain abnormal conditions, however, an altered splenic physiology becomes all-important, so much so indeed that the continued presence of that organ may lead to serious disability and even death. This happens for example in idiopathic thrombocytopenic purpura, in many cases of hemolytic anemia, and in not a few of splenic neutropenia. As in many other pathologic states, the behavior of a disordered organ, in this case the spleen, tends to illuminate the rather more subtle activities of the normal spleen. Thus, in Werlhof's disease, the extreme degree of platelet diminution may be due to the production in excess of a splenic

hormone which controls platelet growth and delivery, i e , idiopathic thrombocytopenic purpura may be a form of hypersplenism.⁴ The leukopenia and granulocytopenia of many different kinds of splenomegaly (e g , cirrhosis of the liver, Felty's syndrome, Boeck's sarcoid, malaria, kala-azar, and the more recently described splenic neutropenia) may be due to the excessive production of another hormone controlling granulocytes. Certainly the marrow is crowded with granulocytes and yet the circulating blood is greatly depleted of these cells. On the other hand, Doan and his group⁵ explain this state of affairs by sequestration and phagocytosis by the spleen. Anemia often occurs with various types of splenomegaly, and certain cases of hemolytic anemia seem to be purely hypersplenic in type, associated as they are with leukopenia and thrombocytopenia, and responding dramatically to splenectomy. Abrami and collaborators⁶ have recently described two types of splenic anemia: a hemolytic form, and one which is purely inhibitory in type. The effects of an abnormal spleen upon the marrow may thus be selective or total, if the latter, pancytopenia or panhematopenia ensues.

These abnormal states of the spleen, particularly splenic neutropenia and pancytopenia, and the great importance of splenectomy as a life-saving measure in these cases are just beginning to be appreciated. Many cases go about unrecognized for months or years, often because the enlarged spleen has not even been palpated. The pancytopenia is usually thought to be due to aplasia or hypoplasia of the marrow, whereas actually the marrow in these states is hyperplastic. Recognition of these cases is facilitated by careful studies and by *correct interpretations* of the clinical, blood, and bone-marrow pictures. It is hoped that continued study will bring these states of hypersplenism more forcibly to the attention of the medical profession.

WILLIAM DAMESHEK

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ABSTRACTS

THE Rh FACTOR AND ITS CLINICAL APPLICATIONS

PHILIP LEVINE
ALEXANDER S. WILNER

Recent Developments in Iso-Immunization by the Rh Factor *Philip Levine Am J Obst & Gynec* 49 810-14, 1945

The author discusses the so-called incomplete or blocking antibody of Race and Wiener. With this reaction and a more recently described slide test of Diamond and its modification by Wiener, it is possible to provide proof of active iso-immunization in almost all instances.

With regard to the complexity of the RhHr system, the author believes that the clinician should think in terms of Rh positive and Rh negative as indicated by the most important anti Rh₀ serum, the only one available for distribution. This serum will aid in detecting 92 per cent of all those potentially immunized by the Rh factor. In order to detect iso-immunization in the remaining Rh positive individuals the blood should be referred to specialists acquainted with the finer subdivision of the Rh and Hr factors and their heredity.

Finally the author stresses the importance of transfusing all Rh negative individuals, especially all of the female population even as infants with Rh negative blood. This measure by itself will reduce the number of erythroblastotic infants and the severity of the condition.—P. L.

On the Hr Factor and the Rh Genetic Theory *Philip Levine Science* 102 1-4 1945

The author presents evidence that the Hr factor is an integral part of the Rh system of variants and its exclusion from any genetic theory is not warranted. Anti Hr sera and anti Rh₁' sera describe three varieties of reactions much like anti M and anti N. A type of blood failing to react with both antibodies does not exist. The view that anti Hr sera are weak because of genetic reasons cannot be accepted. Anti Hr sera are generally weak because of the simple statistical considerations that 92 per cent of Rh negative women produce anti Rh agglutinins while only 2 or 3 per cent Hr negative women can produce anti Hr agglutinins.

Anti Hr sera are of value clinically because they select the homozygous Rh₁Rh₁ where bloods lack the Hr factor.

(The British workers always took into account the Hr factor in their analysis of the genetics of the Rh factor. On a theoretical basis they assumed the existence of three varieties of anti Hr sera to correspond with three varieties of anti Rh sera to give 8 genes [Race *Nature* 153 772, 1944].)—P. L.

Erythroblastosis Fetalis in Mothers with Rh Positive Blood *S. H. Polayes Am J Dis Child* 69 99-102 1945

The author reports two of six cases of iso-immunization and erythroblastosis fetalis in which the mother was in group O and the affected infant in group A. That iso-immunization against the A factor occurred in individuals by a specific increase in the anti A titer. It is implied that the fetus in these cases must be of the nonsecretor type. Iso-immunization by the A factor as well as the finer differences within the Rh-Hr complex explains the origin of erythroblastosis fetalis in the 8 per cent of Rh positive mothers.—P. L.

Coagulation Test for Rh Sensitization *A. S. Wiener J Lab & Clin Med* 30 662-67, 1945

The author produced evidence that the slide test of Diamond depends upon the presence of serum rather than heavy suspensions of blood. The test could be carried out in test tubes with washed blood suspended in normal human serum to make about 2 per cent suspension.

The use of test tubes makes it possible to study the specificity of the blocking antibody which gives agglutination reactions if normal serum is used instead of saline both for suspension of the cells and in titrating the activity of the blocking antibody. At the same time the supply of testing serum is increased.

(Wiener uses the term *conglutination* but the use of this term is criticized by Coombs, Mourant, and Race on the ground that it does not in any way resemble the phenomenon described by Bordet [Brit J Exper Path 1945]. There is still no evidence that this reaction is the basis for Chown's capillary tube test of saline suspended cells.)—P. L.

Prevention of Unintentional Isoimmunization of the Rh Negative Female Population *Philip Levine*
J A M A 128 946, 1945

The author studied the incidence and severity of erythroblastosis fetalis in the first born as influenced by previous immunization with Rh positive blood. He found about twice as many cases in the transfused series, and 60 per cent of these ended in fetal death. In the nontransfused group there was no fetal death and the symptoms were much milder. The possible role of intramuscular injection of Rh positive blood is discussed.

Levine recommends that the entire Rh negative female population requiring transfusion even as infants receive Rh negative blood only. This simple measure should reduce the incidence of erythroblastosis fetalis, especially in its most severe forms.

The biologic test of Wiener is not recommended on the ground that it may serve to immunize the Rh negative individual. (The one fetal death in the nontransfused group could be excluded from the series since the death could be attributed to other factors.)—P. L.

Detection of Weak and Incomplete Rh Agglutinins. A New Test for the Detection of Weak and Incomplete Rh Agglutinins *R. R. A. Coombs, A. E. Mourant, R. R. Race* Lancet, pp 15-16, July 7, 1945

The principle of the test is the use of an anti human globulin serum which will react directly with Rh positive blood previously coated with the incomplete antibody or with weakly agglutinating serum. The test is useful for the detection of fine degrees of sensitization not readily demonstrable by other methods (agglutinins or blocking antibodies).—P. L.

Rh Antibodies *I. Davidsohn* Am J Clin Path 15 95-105 1945

This general review of the subject contains a few noteworthy observations. Davidsohn points out that Rh negative women may be in the negative phase of iso-immunization for a short period following the delivery of an erythroblastic infant. Accordingly, the chances for detecting anti Rh antibodies are best about eight to twenty days following delivery. Presumably at delivery fetal Rh positive blood must be released into the maternal circulation in sufficient quantities to neutralize circulating antibodies.

The observation of Witelsky that anti Rh antibodies are found also in milk is confirmed. The persistence of anti Rh agglutinins following delivery and its influence on the outcome of the next pregnancy are discussed. The observation of Levine that most anti Rh agglutinins belong to the class known as warm agglutinin is confirmed. Although Davidsohn discusses the several varieties of anti Rh agglutinins, he fails to emphasize that the anti Rh₀ serum is clinically the most important variety, which will detect more than 90 per cent of instances of iso-immunization by transfusion and pregnancy.—P. L.

The Rh and Hr Factors in Chimpanzees *A. S. Wiener and M. Wade* Science 102. 177, 1945

The authors make the significant observation that the blood of each of fifteen chimpanzees possessed the Hr factor, but not the Rh factor. Accordingly, they behave like human Rh negative bloods. It is suggested that this uniformity is the result of selective effect of iso-immunization in pregnancy.—P. L.

The Preparation of Potent Anti Rh Typing Serums by Injection of Rh Positive Blood into Previously Isoimmunized Individuals *J. M. Hill, S. Haberman, and A. V. Orozco* J A M A 128 944-46, 1945

The authors injected small amounts (7-10 cc) of Rh positive blood into carefully selected Rh negative patients in order to maintain high levels of anti Rh titers. It is highly probable that much smaller amounts of Rh positive blood will suffice. If this method, carried out on a voluntary basis, were entirely practicable, it would solve the problem of a sufficient supply of human anti Rh diagnostic sera.

By selecting the variety of Rh positive blood to be injected into these patients, it may be possible to produce anti Rh agglutinins of the several varieties —P L

The Rh Factor in Repeated Abortion and Miscarriage *A B Hunt Proc Mayo Clinic 20:28 1945*

The author concludes that iso immunization by the Rh factor does not play an important role in early fetal death. It is only during the latter half of the pregnancy that the iso immunization is initiated and a certain interval of one or more months of intra uterine hemolytic action on fetal Rh positive blood is required before the fetus is affected. Endocrine deficiencies are probably of more importance in habitual abortion.

(The author does not refer to the possible role of iso immunization by the factors A and B in habitual abortion as shown by Levine in the *J Hered 34:71-80 1943*) —P L

The Rh Factor and the Public Health Laboratory *P Lee Frederik A I I an Saun and I I Brown J A M A 128:19, 1945*

This brief report is significant because it represents the first organized effort to carry out prenatal tests as a public health measure on a country wide basis. The list of Rh negative men and women was made available for the formation of a group of Rh negative donors whose blood was used for the treatment of erythroblastotic infants —P L

The Demonstration of Anti Rh Agglutinins—An Accurate and Rapid Slide Test *I K Diamond and N M Abelson J Lab & Clin Med 30:204-12 1945*

Diamond and Abelson describe a procedure which facilitates the detection of the highest incidence of iso-immunization in Rh negative women immunized either by fetal blood or by repeated transfusions. The method consists of use of slides instead of test tubes and heavy suspensions of blood instead of 2 per cent cell suspensions. More specifically, they recommend the mixture on a slide of 0.1 cc of the serum to be tested and 0.1 cc of fresh oxalated blood or a 40-50 per cent washed blood suspension (presumably in saline).

The authors believe that inhibiting, blocking or incomplete antibody as it is variously termed and agglutinins exist independently in most sera. Of 90 sera of mothers of erythroblastotic infants, only 37 had anti Rh agglutinins tested by the incubation technic but the slide test was positive in all. In all but a few, the indirect test for blocking antibodies was positive in the 53 sera which were negative by the incubation technic.

In the presence of an excess of cells 8 to 10 parts of cells and 1 part of serum hemolysis was observed —P L

The Importance of Rh Inhibitor Substance in Anti Rh Serums *I K Diamond and N M Abelson J Clin Investigation 24:122-26 1945*

The authors found that many Rh negative mothers of erythroblastotic infants contain their so-called inhibiting antibodies which are identical with the incomplete antibody of Race and the blocking antibody of Wiener. These antibodies unite with Rh positive blood but the second stage of the reaction i.e., the visible effect of agglutination, does not occur under the usual conditions of testing. These antibodies correspond in specificity to those of anti Rh₀, anti Rh', or anti Rh" agglutinins.

Prolonged heating at 56° inactivates the agglutinin but not the blocking antibody. Of 50 sera of Rh negative mothers recently immunized, 30 had strong agglutinins, 10 had strong inhibiting antibodies, and the remaining 10 had both sorts of antibodies.

Diamond and Abelson report an interesting experiment carried out by injecting an Rh₀ individual with a serum containing inhibiting antibodies. The patient's cells were now 16 times less sensitive to agglutination by anti Rh₀ sera and, significantly, the patient's sera now contained anti Rh₀ agglutinins. The result can be understood if one assumes that serum used for the injection also had anti Rh₀ agglutinins which were masked by the action of the inhibiting antibodies. This was confirmed by the authors by *in vitro* absorption experiments —P L

Individual Blood Differences in Mexican Indians, with Special Reference to the Rh Blood Types and Hr Factor *A S Wiener, J P Zepeda, E B Sonn, and H R Polivka J Exper Med 81:559-71, 1945*

Ninety-eight Mexican Indians were tested for the several agglutinable properties. The results showed

a high incidence of group O (91 per cent) 61 per cent M, 31 per cent N and 78.9 per cent P. All were Rh positive with anti-Rh₀ serum, and the incidence of the subtypes was Rh₁ 48 per cent, Rh₂ 41.8 per cent and Rh₀ 1 per cent. The Hr factor was present in 55.8 per cent. Three individuals of type Rh₁Rh₂ who were Hr negative contained the gene Rh₂ which is so rare in white individuals.—P. L.

Investigations on the Occurrence of Rh Substances in Amniotic Fluid *Ernest Witebsky and James F. Mohn* *J. Exper. Med.* 82, 143-56, 1945.

Witebsky and Mohn present indisputable evidence that, contrary to the views previously held, the Rh factor occurs in a water soluble form. In their studies using amniotic fluid, the authors found considerably less Rh active material than A and B substances in the amniotic fluid of secretors. As with the A and B substances, there are both secretors and nonsecretors, in about the same proportion as with A and B, i.e., about 80 per cent and 20 per cent respectively. There is, however, no correlation whatever of A and B and Rh secretors or nonsecretors.

In three cases of Rh negative mothers of erythroblastotic infants, it is significant that the three Rh positive affected infants proved to be nonsecretors.—P. L.

The Clinical Importance of the Rh Blood Type *L. K. Diamond* *New England J. Med.* 232, 447-50, 1945.

This comprehensive review is based on a large experience with material tested at the Blood Grouping Laboratory, Children's Hospital, Boston. The author stresses the severity of the hemolytic disease in infants if the Rh negative mother was previously immunized by transfusions. The incidence of erythroblastosis fetalis in the author's series is one in 150 full term deliveries. Diamond refers to experimental iso-immunization in ten Rh negative men, six of whom developed satisfactory titers.

(The author does not sufficiently differentiate the varying importance of the several varieties of anti Rh sera, one of which, it was shown, will detect 92 per cent of all instances of iso-immunization. Also, there is no evidence to indicate that the placental barrier must be faulty to permit transplacental iso-immunization).—P. L.

Detection of Rh Sensitization: Evaluation of Tests for Rh Antibodies *L. K. Diamond and N. M. Abelson* *J. Lab. & Clin. Med.* 30, 668-74, 1945.

The authors now believe that their slide agglutination test cannot be elicited with the use of washed blood. The presence of serum rather than a heavy cell suspension is the important factor. The various tests for detection of iso-immunization against the Rh factor are discussed and evaluated. The open slide test is most useful as a rapid screening test for selection of sera containing blocking antibodies.

The highest degree of accuracy is obtained by the several tests (agglutination, blocking, and the use of the serum suspended cells).

(The biological test of Wiener is described, but there should never be any need for this measure, which may serve only to increase the degree of iso-immunization).—P. L.

The Production of Rh Antiserum in Guinea Pigs through Inoculation with Red Blood Cells *B. B. Carter* *Am. J. Clin. Path.* 15, 278-79, 1945.

The production of specific anti Rh agglutinins in guinea pigs injected with human blood is described. Each of the six guinea pigs developed anti Rh agglutinins which after absorption with Rh negative blood gave distinct differentiation of Rh positive and Rh negative blood. The agglutinins corresponded in specificity to the human anti Rh₀.—P. L.

A New Rhesus Antibody *A. E. Mourant* *Nature* 155, 542, 1945.

This antibody, produced by a patient immunized by repeated transfusions, agglutinated 96 per cent of all group O individuals. The 4 per cent nonreactors are mainly Rh₂ or Rh⁺. The author believes that this agglutinin corresponds to the antibody predicted by Fisher, which should fail to react with the cells whose genotype is composed exclusively of Rh⁺, Rh₂, and Rh₃.—P. L.

Anti Hr Serum of Levine *R. R. Race, M. MacFarlane, D. F. Cappell, and R. A. Fisher* *Nature* 155, 54-55, 1945.

On purely theoretical grounds Fisher predicted three varieties of anti Hr sera, called γ , δ , and η . The

St serum seemed to be identical in specificity with Hr sera and to correspond to γ variety. The η variety, reacting with 96 per cent of all bloods, was recently described by Mourant. On the basis of a statement by Waller and Levine the authors believed that the Hr sera of the American workers correspond to the third variety δ .

(The potent anti Hr sera of Levine reacted on about 80 per cent of white individuals and almost all colored individuals. In his series Levine found that in tests with a potent anti Hr agglutinin, the blood of all Rh₁Rh₂ individuals gave positive reactions. The British workers were misled by a clerical error for which Levine assumes full responsibility. Consequently, the third variety δ is still to be described.)—P L

Pathogenesis of Congenital Hemolytic Disease (Erythroblastosis Fetalis) I Theoretical Considerations A S Wiener Am J Dis Child (in press for Feb, 1946)

Some of the puzzles in the pathogenesis of congenital hemolytic disease (and of intragroup transfusion incompatibility) are (1) Why only 1 in 25 to 50 Rh negative individuals exposed to Rh positive blood becomes sensitized to the Rh factor, (2) the lack of correlation between the titer of the anti-Rh agglutinins in the patient's serum and the intensity of sensitization, (3) why certain infants apparently normal at birth suddenly develop jaundice and anemia after several hours or days, often severe enough to cause death, (4) the role of the A and B factors in congenital hemolytic disease, (5) why the first born is almost invariably spared, unless the mother has been sensitized by a previous injection of Rh positive blood, and (6) the occurrence of congenital hemolytic disease when the mother is Rh positive.

1. The author postulates the existence of a pair of allelic genes, K and k , where K confers the capacity to become sensitized readily, while k is the contrasting normal gene. Almost all (about 97 per cent) of individuals belong to genotype kk . Such individuals, if Rh negative, are not apt to become sensitized to the Rh factor after a transfusion of Rh positive blood or after bearing an Rh positive fetus. About 3 per cent of individuals are believed to belong to genotype Kk , and these are the patients who are likely to have erythroblastic infants or intragroup transfusion hemolysis. The rare individuals of genotype KK should be extremely easy to sensitize, and probably account for the rare cases of erythroblastosis in the first pregnancy, or instances of multiple sensitization to Rh and M, etc.

2. Individuals sensitized to the Rh factor may form other varieties of Rh antibodies besides agglutinins. Agglutinins are assumed to be bivalent (having two combining groups) or multivalent, and agglutination occurs as the result of the formation of a latticework (Marrack hypothesis) when the agglutinins combine with Rh positive red cells. Univalent Rh antibodies can be detected only by the use of special techniques: blocking tests or conglutination tests. Univalent antibodies are presumably composed of smaller molecules than agglutinins, and therefore should be capable of traversing the placental barrier into the fetus more readily.

3. The difference between agglutination and conglutination can be summarized as follows:

Rh positive red cells	plus	Bivalent Rh antibodies (agglutinins)		gives Agglutination
Rh positive red cells	plus	Univalent Rh antibodies (blockers and/or glutinins)	plus	Conglutinin (X protein) gives Conglutination

Conglutinin (or X protein) appears to be a complex of serum albumin, serum globulin and phospholipid, which is absorbed by antigen after it has been sensitized by its specific antibody. Because of its colloidal properties X protein causes the sensitized red cells to stick together, and in vivo, probably in conjunction with complement it brings about slow hemolysis. According to the author's theory only the simple precursors of X protein are present in the fetus, but the physiologic changes occurring after birth cause these to aggregate into the larger molecules of conglutinin, thus accounting for the delayed onset of congenital hemolytic disease.

4. The natural A and B agglutinins are believed to be composed of large molecules. Only when individuals of genotype Kk (or KK) become sensitized are univalent A and/or B antibodies formed, which can traverse the placenta more readily. Congenital hemolytic disease occurring in this way may be indistinguishable clinically from cases caused by Rh sensitization, usually, however, the A-B-O cases are milder.

5. During pregnancy villi may become detached, but the fetal red cells contained in them will usually

be too few in numbers to sensitize any but the rare patients of genotype AA. During labor and delivery owing to the disturbances at the placental site presumably larger numbers of fetal red cells could gain access to the maternal circulation sufficient also to sensitize patients of genotype AA.

6 These cases are explained by sensitization to the A B factors as indicated under (4) and by sensitization of mothers of one Rh blood type to blood of a different Rh type or sensitization to the Hr factor etc. Inasmuch as factors Rh' Rh", and Hr are much less antigenic than Rh₀ these cases are rare — A S W

Pathogenesis of Congenital Hemolytic Disease (Erythroblastosis Fetalis) II Illustrative Case Histories of Rh Sensitization *A S Wiener and E B Sonn* Am J Dis Child (in press for Feb 1946)

Eleven cases are presented which illustrate the following points

1 The Hr test is a valuable aid in determining the homozygosity or heterozygosity of type Rh₁ individuals. When the Hr test is negative the individual is homozygous (either genotype Rh₁Rh₁ or Rh₁Rh₀) while if the Hr test is positive he is almost surely heterozygous. If a man is homozygous for the Rh factor and his wife's serum contains Rh blocking antibodies the prognosis for future pregnancies is virtually hopeless.

2 In the presence of Rh sensitization, plasma transfusions should be given instead of blood transfusions unless Rh negative donors are available.

3 Even a small intramuscular injection of Rh positive blood may be sufficient to sensitize an Rh negative woman and thus prevent her from having viable infants.

4 The injection of a potent antigen may prevent an individual from becoming sensitized to a weaker antigen to which he or she is exposed simultaneously. For example the injection of typhoid or pertussis vaccine during pregnancy may be worth while in the case of Rh negative patients with sisters who have had erythroblastic infants. Once sensitization is developed however counterimmunization does not appear to affect the degree of sensitization.

5 That univalent antibodies (blockers) are more important than bivalent antibodies (agglutinins) in the pathogenesis of congenital hemolytic disease is proved by the ease with which the former can be demonstrated in the serum of erythroblastotic infants in the absence of agglutinins.

6 In many cases early cesarean section is ineffectual in the prevention of congenital hemolytic disease because the disease often is initiated by the birth of the infant. According to the author's theory the profound physiological changes occurring after birth cause the formation of γ protein in the infant's plasma and thus favor the onset of hemolysis. Besides cesarean section subjects the mother to the additional hazards of an operation and the infant to the hazards of prematurity.

7 The presence of high hemoglobin concentration in an infant with hemolytic disease may merely mean that the disease process is mild but frequently such cases develop kernicterus or hemorrhagic manifestations and die. In any event there is nothing to be gained by prophylactic transfusions before the hemoglobin drops below 80 per cent because the only effect of transfusion therapy is to correct the anemia and the only infants benefited are those who would otherwise die from anemia. In any case, transfusion of too much blood is to be avoided because this is dangerous.

8 Women differ in the ease with which they can be sensitized to the Rh factor so that while most require only one or two pregnancies or transfusions occasionally sensitization does not develop until there have been as many as seven to ten or more pregnancies. Once sensitization has developed it appears to be permanent though patients differ in the degree of sensitization. Thus some patients may have repeated stillbirths while others have viable infants who recover after transfusion therapy.

9 A woman developed high titered anti Rh₀ and anti Rh" agglutinins after giving birth to a type Rh infant who died of hemolytic disease. The titer of the antibodies showed hardly any change after thirteen exchange transfusions with normal Rh negative male donors. The husband was shown to be homozygous for the Rh factor, so when the patient became pregnant a therapeutic abortion was performed.

10 For transfusing erythroblastotic infants fresh blood is preferable to bank blood when available. Improperly stored blood breaks down quickly in the infant's circulation.

11 Mothers with latent diabetes may have an obstetrical history clinically indistinguishable from erythroblastosis fetalis. In one such case the difficulty of diagnosis was increased by the fact that the patient was Rh negative and the husband Rh positive. In such instances testing for Rh antibodies on the maternal serum by the conglutination method is a reliable aid in the differential diagnosis — A S W

NEWS AND VIEWS

PERSONALIA

On the evening of December 5, 1945, Dr George Richards Minot of Boston was tendered a testimonial dinner on the occasion of his sixtieth birthday. Many distinguished colleagues who had been associated with Dr Minot and his work were present. Dr William B Castle as toastmaster introduced the speakers, who discussed various stages of Dr Minot's activities, from his childhood on. The speakers included Dr Henry A Christian, Dean C Sidney Burwell, Dr Elliot P Joslin, Dr F M Rackemann, Dr J H Means, Dr James B Manary, and Dr Lawrence B Ellis.

Dr Reginald Fitz on behalf of the President and House of Delegates of the American Medical Association presented Dr Minot with a medal conferred for distinguished service to the profession.

The toastmaster presented Dr Minot with a bound copy of letters of congratulation from distinguished leaders in the field of hematology and clinical investigation, both in America and abroad.

As a climax to the occasion Dr Minot was notified of his election to membership in the Academy of Medicine of Paris.

Dr Emil Schwarz, formerly of Vienna, Austria, and now working in the Hematology Department of the Michael Reese Hospital, Chicago, has recently celebrated both his eightieth birthday and the receipt of his United States citizenship. He is reportedly the oldest person to have attained citizenship recently. His activities and continued keen interest in the field of hematology belie his age.

Dr William Dameshek of Boston, upon invitation of the National University of Mexico, gave a series of lectures at the Medical School in Mexico City in January. The titles of the lectures were as follows: Physiologic Principles in Anemia, Hemolytic Anemia, and Certain Disorders of the Spleen. Professor I Gonzalez-Guzman, Dean of the Medical School, presided. At the conclusion of the lectures, Dr Dameshek was awarded an Extraordinary Professorship of Medicine in the Medical School.

Dr Moises Grinstein, of Córdoba, Argentina, is joining the staff of the School of Medicine of the University of Utah to work in the field of porphyrins. Several years ago, Dr Grinstein worked with Dr C J Watson at Minnesota. The Utah

group has become very much interested in the porphyrins as well as in the metabolism of iron and copper in connection with their studies of the nature of anemia of infection

Dr Steven O Schwartz has recently been appointed Hematologist to the Hektoen Institute for Medical Research, of the Cook County Hospital Dr Schwartz will continue in his present capacity as Director of the Hematology Laboratories of the hospital

Dr Thomas Benton Cooley (Cooley's anemia) died on October 13, 1945, in Detroit A graduate of the University of Michigan, Department of Medicine and Surgery, Ann Arbor, 1895, he was born in Ann Arbor, Michigan, June 23, 1871 He was professor and head of the Department of Pediatrics at Wayne University Medical School from 1936 to 1941, when he became emeritus professor He was Past President of the American Pediatric Society, and of the American Academy of Pediatrics, Founder President of the Detroit Pediatric Society, Past President of the Detroit Academy of Medicine For many years he was chief of the pediatric service and chairman of the staff of the Children's Hospital in Detroit He died at the age of seventy-four, of hypertensive heart disease

MISCELLANEOUS

The Massachusetts Department of Public Health will soon begin distribution of blood plasma and other blood products without cost on the same basis as serums and vaccines The service will operate as a unit of the division of biologic laboratories The legislature has appropriated \$174,000 to equip, staff, and operate a blood and blood derivatives program, and the Godfrey M Hays Trust of Boston has donated \$176,000 to Harvard University to construct a modern, well equipped laboratory building in which processing and fractionation of blood and its products can be carried out The project will include collection of blood, processing of the blood, and distribution of these products

The Medical Society of the County of New York has incorporated a nonprofit organization known as the Blood and Plasma Exchange of New York, Incorporated, to make blood and plasma readily available to patients in civilian hospitals at a small cost

The Mexican cardiological and hematological journal, *Archivos Latino-americanos de Cardiología y Hematología*, which has been published since November, 1930, has discontinued publication The hematological section of the journal was under the editorial direction of Professor Ignacio Gonzalez-Guzman of Mexico City With the inauguration of the new National Institute of Cardiology in Mexico in 1944, the journal became devoted strictly to cardiology, beginning with the October, 1944, number The present name of the journal is *Archivos del Instituto de Cardiología de México*, and its Editor-in-Chief is Dr Chavez

The French hematological publication, *Le Sang*, has been issued regularly throughout the war under the editorship of Paul Chevallier and published by G

Doin & Company of Paris Beginning with the fourth number of the sixteenth volume published in November, 1944, the review is under the direction of the following Editorial Board Editor-in-Chief, P Emile-Weil, Associate Editors, Noel Fiessinger, Paul Chevallier, J Koskam, Editorial Secretary, Jean Bernard

A number of reports, mostly unofficial, indicate that folic acid may well turn out to be the chemical of the year This particular fraction of the vitamin B complex is probably not itself the long searched for liver extract factor, although there seems to be little doubt that it induces typical reticulocyte and erythrocyte responses in cases of Addisonian pernicious anemia However, we learn that the most brilliant results have thus far been obtained in the treatment of sprue, and in other macrocytic anemias related to but not identical with pernicious anemia It is expected that complete data on the use and limitations of folic acid will shortly be available

SOCIETY

The first meeting of the recently established Society for the Study of Blood was held at the New York Academy of Medicine on June 14 Addresses were delivered as follows Development of Hematology in New York City by Dr Nathan Rosenthal, The Role of the Hematologist in the General Hospital by Dr Eugene R Marzullo, and Blood Banks of the Future by Dr Lester J Unger The officers elected are President, Dr Alexander S Wiener, Vice-President, Dr Paul Reznikoff, Secretary-Treasurer, Dr Peter Vogel A second meeting was held at the New York Academy of Medicine November 8 to hear the following speakers Dr John T Edsall, Boston, on The Anti-hemophilic Globulin of Normal Plasma, Dr Louis K Diamond, Boston, on The Clinical Use of an Antihemophilic Fraction of Blood Plasma, and Dr Randolph West, New York, and Erwin Chargaff, Ph D, New York, on Hemophilic Clotting Defect in a Female

BOOK REVIEWS

Hematology for Students and Practitioners By WILLIS W FOWLER M D Paul B Hoeber, Inc., New York
London Pp 499 \$8 00

For a long time, there was a dearth of American texts dealing with the blood. In the last few years however this deficiency has rapidly been remedied and it is probable that before long the pendulum will swing the other way. The most recent of the new texts is that by Fowler of the University of Iowa. The book originated as an expansion of the author's lecture notes for medical students, and as such is characterized by simplicity of presentation. It often seems too simple, however even for those who are relatively uninformed. This being so, the practitioner who wants some real information on a given subject had better turn to more comprehensive texts.

There are many things in the book with which one might take issue. For example the megaloblast is pictured as the precursor of the normal nucleated red cell (normoblast); many investigations have shown that it is an abnormal cell characteristic of a liver extract deficiency. The colored plates dealing with the megaloblasts are poorly done as are those of the platelets. Much of the text is old fashioned and outdated by recent reports, e.g. the description of so-called Lederer's anemia, and its supposed specific response to transfusions, the supposed effectiveness of pentose nucleotides in agranulocytosis with no mention of the value of antibacterial agents such as the sulfonamides or penicillin, the use of the term Banti's syndrome, the lack of any discussion of the milder forms of Mediterranean anemia etc., etc.

The discussions dealing with the deficiency anemias are well presented, and the chapter on the Myelophthitic Anemias is reasonably well done. The bright spot of the book, however is the chapter by DeGowin on transfusions of blood and blood derivatives. This stands out because it is refreshingly up-to-date, complete and excellently written. There is a short chapter on hematologic methods. An outstanding physical characteristic of the book is the excellence of the print work which is done on high grade glossy paper.

Blood Groups and Transfusion By ALEXANDER S WIENER C C Thomas Co., Springfield, Ill., 1945 3rd edition second printing Pp 438 \$7 50

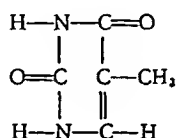
Since the first edition of this now standard and well known text was published a decade ago, the subject of the blood groups and transfusion has attained a rapid and truly monumental growth. This is due chiefly to two factors: the war, with its emphasis on transfusions of blood, plasma and plasma fractions, and secondly the startling discovery of the Rh agglutinin and its relationship to intra-vascular transfusion reactions and to acute hemolytic anemia of the newborn. These developments in which Wiener has been a leading figure have necessarily led to extensive revisions and additions as a result the text is almost twice as long as in the first edition. Some of the older material dealing with transfusions of blood might well have been omitted and more space given to the Rh factor and to the all too short chapter on Blood Groups and Disease. For example very little is said about the cold hemagglutinin and its relationship to primary atypical pneumonia. This agglutinin is not infrequently the occasion for the diagnosis of incompatibility and may be responsible for certain cases of hemolytic anemia and of gangrene. The subject of hemolysins is not discussed although since complex hemolysins and agglutinins are closely related, it might conceivably have been given a place in this volume. These criticisms it must be confessed are perhaps made because of a personal bias on the part of the reviewer, and should by no means detract from the value of this book which is outstanding. Its importance as a standard reference text particularly from hereditary and medico-legal aspects, has been thoroughly proved.

ANTI-ANEMIC PROPERTIES OF THYMINE

By TOM D SPIES, M D , WALTER B FROMMEYER, JR , M D , CARL F VILTER, M D ,
AND ANN ENGLISH, B S

THYMINE (2,4-dioxo-5-methyl pyrimidine), one of the nucleotides found in thymo-nucleic acid, was isolated by Kossel and Neumann in 1893,¹ and a few years later was synthesized by a number of other investigators. Since it is an integral portion of the biologic cell we have had special interest in the possibility of its having a role in hemopoiesis for some time. Our recent studies² showed that no hemopoietic response in macrocytic anemias occurred following the administration of one gram or less of thymine daily and that even 4.5 grams given daily produced only a submaximal response. The present communication is concerned with the effect of administering larger doses of thymine to patients with Addisonian pernicious anemia in relapse.

Thymine (2,4 Dioxo-5 methyl pyrimidine)



Three patients with macrocytic anemia and a histamine refractory achlorhydria and achylia were admitted to the hospital for study. The diet, which excluded all meat, meat products, fish, poultry, and uncooked fresh leafy vegetables and which allowed only one pint of milk and one very well cooked egg a day, was rigidly controlled. Daily hematological studies were performed as previously described.³

Physical examination on admission in all three subjects revealed the characteristic signs of pernicious anemia and, in addition, arteriosclerosis of moderate degree. One of the patients had a chronic pelvic infection which was active during the first eleven days of her treatment period. The other two subjects had no evidence of acute or chronic infection.

Prior to treatment a complete blood count, blood indices, sternal bone marrow studies, icterus index, urinalysis, stool analysis, gastric analysis, oral glucose tolerance tests and gastro-intestinal X-rays were made on each patient. In each of the three cases the color index was above 1.0, and the mean corpuscular volume was greater than 110.0 cubic microns, the mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were greater than 32 micro-micrograms and 34 per cent, respectively. Sternal bone marrow was obtained by means of the Turkel trephine, the puncture being made through the sternum opposite the second

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intercostal space. The bone marrow preparation from each patient showed arrest of the cells at the megaloblastic level. In each case the icterus index was above normal and the urine and stools were essentially negative. Gastric analysis after histamine stimulation showed achlorhydria and achylia in all the patients. In each case, upper gastro-intestinal series and oral glucose tolerance tests were essentially normal.

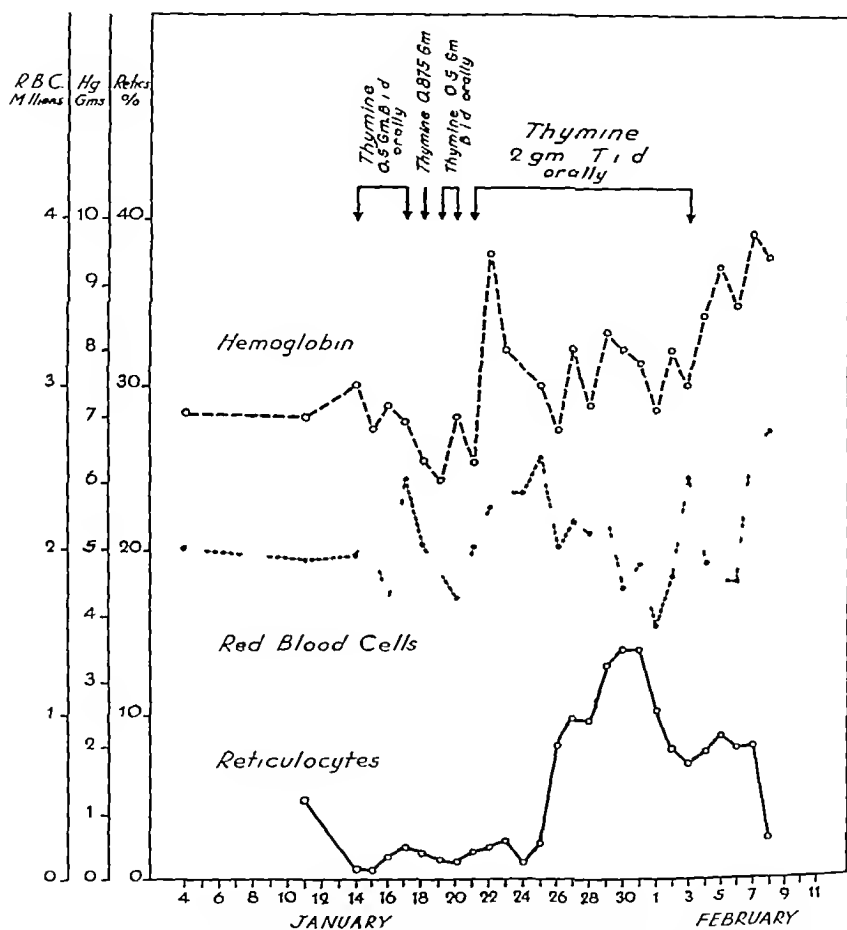


FIG. 1. THE HEMATOLOGIC RESPONSE IN A PATIENT WITH PERNICIOUS ANEMIA IN RELAPSE TO VARYING DOSES OF THYMINE

The thymine was weighed on an analytical balance and suspended in one-half glass of cold tap water. The glass was rinsed twice with water which the patient drank in order to insure his obtaining any undissolved thymine which might cling to the sides of the glass.

Case I was given 0.5 grams of thymine twice daily for six days without any hematological or clinical response. The dose was then increased to 2.0 grams three

times a day for fourteen days The effectiveness of these dosages is shown in Fig 1

Case II received 3 4 grams three times a day for eleven days

Case III was given 1 5 grams three times a day for fourteen days The dose was then increased to 3 4 grams three times a day The response of these three patients to this therapy is shown in the accompanying tables

In Case I the initial reticulocyte response and the peak reticulocytosis were delayed This might have been due to an acute exacerbation of a chronic pelvic

TABLE 1 —Effect of Oral Administration of Thymine on Peripheral Blood in Pernicious Anemia

Case	R B C (millions per cu mm)			Hg (Gm per 100 cc)			Reticulocytes (%)			Dosages thymine			Remarks
	Initial	14 days	Final day	Initial	14 days	Final day	First day of rise	Day of peak	Per cent at peak	Daily dose (Gm)	No of days	Total Gm	
I (B B)	2 01	2 43	2 88 (22)	6 3	7 5	10 4 (22)	6	11	14 2	6 0	14	84	59 year old Negro female Chr pelvic inflammatory disease Moderate arteriosclerosis Received 1 0 Gm q d X 6 without response
II (H R)	1 58	2 29	2 51 (17)	6 2	10 1	9 8 (17)	4	6	19 0	10 2	11	112 2	57 year old white female Moderate arteriosclerosis
III (D L)	1 67	1 93	2 34 (18)	7 5	9 4	9 8 (18)	6	11	9 0	4 5 10 2	13 4	58 5 40 8	75 year old white male Moderate arteriosclerosis

TABLE 2 —Effect of Oral Administration of Thymine on Leucocytes in Patients with Pernicious Anemia

Case	Initial	7th day	14th day
I (B B)	2950	4950	9200
II (H R)	4200	5200	6100
III (D L)	6950	7750	8950

infection during the first eleven days of therapy Case II showed the type of reticulocyte response which could be expected following adequate therapy with a potent liver extract ^{4 5} In Case III an effort was made to determine the optimal dose by means of the double reticulocyte response described by Castle and Minor ⁶ After what appeared to be a submaximal reticulocyte response to 1 5 grams of thymine three times a day and after the reticulocytes were consistently decreasing in number, the dose was increased to 3 4 grams three times a day The second reticulocyte peak of 8 8 per cent occurred on the 6th day after the increase of dosage Coinci-

dent with the reticulocyte response in each case, there was a great increase in appetite, strength, and vigor

Sternal bone marrow studies were repeated on the 17th, 7th, and 12th days after treatment was instituted in Cases I, II, and III respectively. In Cases I and II the bone marrow smears showed a reversion from bone marrow characteristic of megaloblastic arrest to bone marrow which was normoblastic. Such a change was indicative of adequate therapy with a potent anti-anemic substance and a reversion toward a normal bone marrow. The bone marrow in Case III did not show as marked a normoblastic reversion as in Cases I and II. Although some megaloblastic arrest was evident, there was a definite increase in the number and percentage of normoblasts with a concomitant decrease in the number and percentage of megaloblasts as compared with the control bone marrow smear. Thus, the marrow in this case was characteristic of an inadequately treated case of erythrocyte maturation factor deficiency anemia.

SUMMARY

Three patients with Addisonian pernicious anemia were selected, hospitalized and given a diet devoid of meat and meat products. After baseline hematological studies were made and checked repeatedly, daily large doses of thymine were given orally.

The clinical and hematological improvement in these three patients was in every way similar to that which follows the administration of folic acid to patients with pernicious anemia in relapse. The exact mode of action of thymine is obscure, but it is possible that folic acid may act as an enzyme or co-enzyme in the synthesis of thymine or a thymine-like compound. Such synthesis may take place in the gastro-intestinal tract. The present findings indicate that thymine has anti-anemic properties and a profound effect on the general metabolism of patients with Addisonian pernicious anemia in relapse.

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ACUTE HEMOLYTIC ANEMIA AND HEMOGLOBINURIA FOLLOWING SULFADIAZINE MEDICATION

REPORT OF A CASE

By JOSEPH F. ROSS, M.D., AND BERTHA L. PAEGEL, M.D.

ACUTE hemolytic anemia is a serious complication of medication with sulfanilamide and its derivatives. Its incidence varies with the type of sulfonamide employed, being highest with sulfanilamide and lowest with sulfadiazine. It is important, however, to realize that no sulfonamide compound is free from the hazard of producing acute hemolytic anemia, and to recognize the grave nature of this complication. Death has occurred in between 5 per cent and 10 per cent of the reported cases of sulfonamide hemolytic anemia. The importance of promptly recognizing the signs and symptoms of acute intravascular hemolysis, and the necessity for immediately instituting proper therapeutic measures can scarcely be overemphasized.

The mechanism by which the hemolysis is produced is obscure. We are reporting the findings in a case of acute hemolytic anemia which appeared following the administration of sulfadiazine, since certain abnormalities in the erythrocytes give some indication of the nature of the hemolytic process. Seven cases previously have been reported in which acute hemolytic anemia is stated to have been produced by sulfadiazine.¹⁻⁵ However, in several of these cases the etiologic role of sulfadiazine is very questionable.

CASE REPORT

K. P., a 4 year old, white female child, was admitted to the Pediatric Service of the Massachusetts Memorial Hospitals at 5:30 a.m. July 22, 1944, because she had been voiding dark, bloody urine during the past 19½ hours. Five days prior to admission she developed a severe sore throat and a non-productive cough. Two days before admission her temperature was found to be 103° F. and a physician was called to see her. He found evidences of tonsillitis and pharyngitis and recommended that 0.25 gram of sulfadiazine be given every four hours. The first dose of 0.25 gram of sulfadiazine was given at 4:00 p.m. on July 20, 1944, and the child immediately vomited. A second dose of 0.25 gram was given four hours later and she again vomited. No more sulfadiazine was given until 8:00 a.m. the next day, July 21, 1944, when a third dose of 0.25 gram was administered and was again followed by vomiting. No more sulfadiazine was given. The child was weak, pale and listless, and at 10:00 a.m. voided urine which was of a dark, bloody color. Large amounts of fluid were given by mouth and there was resultant frequency of urination. All urine was of a dark, bloody appearance. The child became progressively weaker and finally fainted in the bathroom one hour before admission to the hospital.

She had received a total of 0.75 gram of sulfadiazine, much of which was vomited immediately after ingestion. The first dose was given 37½ hours before and the last dose 22½ hours before admission.

The child had received sulfonamide medication on two previous occasions.

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3 years before the present illness she was given sulfathiazole in small amounts for a period of two days because of a cold and bronchitis. She vomited several times immediately after receiving the drug but showed no other ill effects.

2 years before the present illness she received 0.25 gram of sulfadiazine every four hours for a period of two days because of a severe chest cold. There was no reaction to this medication.

The patient had been a normal full term baby and had developed normally. She had been immunized against diphtheria and whooping cough. She had not contracted any of the childhood diseases, and except for three episodes of upper respiratory infections and bronchitis 3 years, 2 years and 10 months before the present illness had always been well. There had been no evidence of allergy. The family history was negative. Her mother had been born in Ireland and her father was a native born American of Portuguese extraction. One male sibling aged 2 years was well.

Physical examination at the time of admission to the hospital revealed a well developed well nourished white female child appearing acutely ill and lethargic. The temperature was 100.8° F per rectum. The pulse was of poor quality and the rate was 148 per minute. The respiratory rate was 26 per minute. The skin was warm, dry, pale and slightly icteric. There were no petechiae and no rash. The ear drums were negative. The sclerae were icteric. The eyes were not otherwise remarkable. There was no nasal discharge. The mucous membranes were very pale. The teeth were normal. The tonsils were hypertrophied and moderately injected. The pharynx was slightly injected but there was no exudate. The neck was supple and there was no enlargement of the lymph nodes in the cervical region or elsewhere. The thorax was not remarkable and respiratory expansion was normal. The lungs were clear to percussion and auscultation and no rales were heard. The heart was of normal size. The rhythm was regular and the rate 148 per minute. The sounds were of poor quality. No murmurs were heard. The systolic blood pressure was 100 millimeters of mercury. Korotkoff's sounds were audible at zero pressure. The abdomen was soft, symmetrical and non tender. No masses were palpable. The liver, spleen and kidneys could not be felt. There was no costovertebral tenderness. The external genitalia were normal. There was no peripheral edema and no redness, swelling or tenderness of the joints. Reflexes were physiological. Her weight was 35 pounds, and her height 39 inches.

Laboratory Findings. Urine voided shortly after admission was deep red in color, and after centrifugation the supernatant urine was of the same color and gave a strongly positive benzidine reaction. Spectroscopic examination revealed the characteristic absorption spectrum for oxyhemoglobin. No methemoglobin was found (table 1). The slightest possible trace of albumin was present but there was no sugar, ketone or bile. Urinary sediment contained 3-5 leucocytes, many red blood cells and an occasional granular cast per high power field. The erythrocyte count was 2,220,000 per cubic millimeter, the hemoglobin 6.0 grams per cent, and the leucocyte count 43,000 per cubic millimeter. Differential leucocyte count was as follows: Polymorphonuclear neutrophilic leucocytes 80 per cent, lymphocytes 12 per cent, monocytes 5 per cent, eosinophils 3 per cent. Blood studies are summarized in table 2. The blood serum was deep red and contained a large amount of hemoglobin. Subsequent spectroscopic examination revealed the absorption spectra of oxyhemoglobin and methemalbumin (table 3). The blood non protein nitrogen was 31 mg. per cent. The carbon dioxide combining power of the plasma was 47.5 volumes per cent. Blood obtained at the time of admission contained 0.84 mg. per cent of sulfadiazine per 100 cc., and the following day no sulfadiazine could be demonstrated. Blood culture taken on admission showed no growth. A culture of the throat revealed numerous colonies of hemophilus influenzae and hemolytic staphylococcus aureus (coagulase positive), a few colonies of pneumococcus type 19, micrococcus flavus, micrococcus catarrhalis and diphtheroid bacilli. No hemolytic streptococci were recovered. Two stools were well formed, brown and the guaiac test was negative. The Hinton test for syphilis was negative. The patient's blood was group O, Rh factor positive. The estimated total blood volume was 1430 cc.

Clinical Course. The clinical course is summarized in Figures 1 and 2.

July 22, 1944. On admission the child appeared to be on the verge of vascular collapse, but with rest her condition gradually improved. An infusion of 400 cc. of 10 per cent glucose in normal saline solution was given without marked response. Her erythrocyte count had fallen to 1,820,000 by 8:00 p.m. Transfusion of 450 cc. of citrated blood produced considerable clinical improvement and increased the erythrocyte count to 2,680,000 per cubic millimeter. Six grams of sodium bicarbonate were given by mouth during the course of the day and the pH of the urine was 8. Marked hemoglobinuria continued all day as noted in table 1, but the urinary output was normal. She was given 5000 units of penicillin intramuscularly every three hours during the first five days of her hospital stay.

July 23, 1944 Hemoglobinuria continued all day, and the erythrocyte count which had been 2,680,000 at 11 30 p m after the transfusion on the previous day had fallen to 1 600,000 at 8 00 a m The patient was again transfused with 225 cc of citrated blood with resultant improvement in color and elevation of the erythrocyte count to 2,130,000 at 8 00 p m Following the transfusion the urine became lighter in color but still contained hemoglobin No sulfadiazine could be detected in the blood Four grams of

TABLE I—Urinary Hemoglobin Excretion

Date	Time	Volume	Color	Abnormal Urinary Pigments				Sediment per high power field
				Conc n Total Heme Pigment	Absorption spectrum char acteristic of		Total Heme Pigment Excreted	
					Oxy hemo- globin	Met hemo- globin		
7/22/44 to 7/23/44	5 30 A.M. to 8 00 A.M.	700	Deep sherry red	mg % 241	++++	None	mg 1 690	Many RBC 3-4 WBC occasional granular casts
7/23/44 to 7/24/44	8 00 A.M. to 8 00 A.M.	740	Amber	36	+	None	268	Rare RBC 5-6 WBC 2-6 hyaline and granular casts
7/24/44 to 7/25/44	8 00 A.M.	210	Deep sherry red	84	+	None	177	
	10 45 A.M.	105	Burgundy red	154	+++	++	162	
	3 15 P.M.	90	Deep burgundy red	202	++++	None	182	
	5 00 P.M. to 8 00 A.M.	280	Deep reddish brown	69.3	+	+	194	
24 hr amt		685					715	
7/25/44	8 00 A.M. to 7 00 P.M.	50	Brown	60	+	None	30	Many WBC many granular casts
7/25/44 to 7/26/44	7 00 P.M. to 7 00 A.M.	165	Amber	35	±	None	58	
23 hr amt		215					88	
7/26/44 to 7/27/44	7 00 A.M. to 7 00 A.M.	450	Amber	15	None	None	67	
7/27/44 to 7/28/44	7 00 A.M. to 7 00 A.M.	850	Amber	2.4	None	None	22	Negative

sodium bicarbonate were given during the course of the day and an alkaline reaction of the urine was maintained. The urinary output remained normal.

July 24 1944 The erythrocyte count had fallen to 1 320,000 and the urine, which had tended to clear in the evening of the preceding day again became port wine in color and contained large quantities of hemoglobin. It appeared that the hemolytic process was still active even though the last dose of sulfadiazine had been given 72 hours previously and no sulfadiazine could be detected in the blood at this time.

TABLE 2

Blood Morphology	7/22/44			7/23/44			7/24/44		7/25/44		7/26/44	7/28/44	8/1/44	9/12/44	10/16/44	10/8/45
	6 A M	7 P M	11 30 P M	8 A M	2 P M	8 P M	9 A M	9 P M	9 A M	3 30 P M						
Erythrocytes, M/cu mm	2 22	1 82	2 68	1 60		2 13	1 32	4 63	4 64	4 82	4 46	3 97	4 35	5 81	5 15	4 63
Hemoglobin, Gm %	6 0			4 2			4 0		12 3	12 7	12 1	11 2	11 3	14 8	14 4	12 9
Hematocrit, %	18 0			12 5	12 0		12 0		37 0	37 2	36 4	34 1	34 1		42 4	40 3
M C V, cu μ	80 5			78 1			91 0		79 7	77 2	81 6	85 9	78 4		82 3	
M C H, %	27 0			26 3			30 3		26 5	26 3	27 1	28 2	26 0	25 3	28 0	
M C H C, %	33 3			33 6			33 3		33 2	34 2	33 1	32 9	33 1		34 0	
Nucleated red cells																
per cu mm							7,920				0	0	0	0	0	0
% total RBC							0 6									
Reticuloocytes																
per cu mm							71,280		134,960		200,700	142,920	95,700	0	0	0
% total RBC							5 4		2 8		4 5	3 6	2 2	0	0	0
Spherocytes																
per cu mm							617,760				892,000	746,360	330,060	0	0	0
% total RBC							46 8				20 0	18 8	7 6	0	0	0
Anisocytosis	+++			+++			+++				+++	++	+	0	0	0
Poikilocytosis	+++			+++			+++				++	+	+	0	0	0
Polychromasia	+++			+++			+++				+	0	0	0	0	0
Leucocytes per cu mm	43,000			38,000			63,650				++	0	0	0	0	0
Neutrophils, %											27,225	12,800	7,025	9,250	6,025	9,300
Adult	70			44			53		75		70			33	29	25
Band	10			16			15		8		9			3	0	0
Metamyelocytes																
Myelocytes	0			6			4		1		0			0	0	0
Lymphocytes, %				0			4		0		1			0	0	0
Small	8			20			15		6		10			46	56	63
Large	4			12			4		3		4			7	5	0
Monocytes, %																
Adult	5			2			1		3		2			5	5	4
Young	0			0			2		3		1			2	1	1
Eosinophils %	3			0			1		1		2			1	4	5
Basophils %	0			0			1		0		1			3	0	2
Platelets per cu mm							218,000				413,000	234,000	192,000	146,000	253,000	232,000
Flood Chemistries																
N P N mg %	31			31												
CO ₂ combining power vol %	47 5			49 4												
Sulfadiazine level mg %	0 84			0												

The patient was seen by us for the first time on this date, and investigation of the nature of the hemolytic process was instituted. No abnormal hemagglutinins or hemolysins were found in her serum at any time (table 4). Erythrocytes obtained shortly after admission and on subsequent days were studied for abnormal sensitivity to cold, warmth, lowered pH and hypotonic solutions of sodium chloride (table 5). The only abnormality detected was an increased susceptibility of the red blood cells obtained July 24th to hemolysis when exposed to hypotonic solutions of sodium chloride. Erythrocytes obtained after this date showed normal resistance to hypotonic saline. The fragility curves are graphically presented in Figure 3. This increased hypotonic fragility was undoubtedly a reflection of the large numbers of spherocytes present in the blood.⁶ Examination of the blood smear showed that 46.8 per cent of all red blood cells were spherocytes (table 2). There was extreme variation in the size and shape of the erythrocytes and polychromasia was marked. Reticulocytes were numerous and a few nucleated red blood cells were present (table 2).

The leucocyte count which had been high on admission, reached a level of 63,650 per cubic millimeter on this day, and myelocytes and metamyelocytes were present in the peripheral blood (table 2).

TABLE 3 —Blood Serum Pigments

Date	Heme Pigments				Bilirubin	
	Total Conc n	Absorption spectrum characteristic of			Direct	Indirect
		Oxy hemoglobin	Methemalbumin	Methemoglobin		
	mg %					
7/22/44		++++	+	None	1 43	4 34
7/23/44		++++	++	None	2 38	4 32
7/24/44	213	++++	+++	None	1 66	3 95
7/25/44	192	++++	++	None	1 62	3 74
7/26/44	145	+++	+	None	1 81	4 56
7/28/44	74	++	+	None	0 32	0 90
8/ 1/44	8	None	None	None	0 08	0 22

At 4:00 p.m. a vein in the left ankle was cannulated and 1100 cc. of citrated blood were administered during the next eight hours. Very marked objective and subjective improvement occurred during the course of the transfusion and at its conclusion the erythrocyte count had risen to 4,630,000.

July 25 to August 1, 1944. Following the 1100 cc. transfusion, the hemolytic process was arrested and the erythrocyte count, the hematocrit and the hemoglobin concentration were maintained at satisfactory levels. However, abnormal heme pigments persisted in the blood serum for the next three days (table 3). The characteristic absorption spectra of oxyhemoglobin and of methemalbumin were detectable until July 29. The latter pigment was detected in all specimens of serum from July 22 through July 29 and was identified by its absorption maximum between 623 m μ and 624 m μ , and by the failure of 10 per cent ammonium sulphide to disperse this absorption band, although it was readily dispersed by concentrated ammonium sulphide. Spectroscopic examination showed that neither the serum nor erythrocytes contained methemoglobin or sulfhemoglobin.

Hemoglobin was not visible in the urine after the transfusion on July 24, although small quantities were detectable by the hemochromogen technic on July 25 and July 26.

Following the transfusion of July 24 and the restoration of relatively normal blood levels of erythrocytes and hemoglobin, the child appeared quite well. Her fever rapidly subsided (fig. 1), she was allowed out of bed on July 27, and was discharged home on August 1, apparently none the worse for her illness.

At no time during the course of the illness had there been evidence of pneumonitis of any type.

Blood studies made on August 1 showed only a mild anemia, normal leucocyte and differential counts, and a normal platelet count. The erythrocytes were practically normal in appearance and only a few spherocytes were noted on the smear. The urine was completely normal.

September 12, 1944. The patient returned for blood studies. She had been well since discharge and had gained weight. No abnormalities in the urine had been noted. Physical examination revealed enlarged

and injected tonsils. Heart and lungs were not remarkable. Liver and spleen were not palpable. The remainder of the examination was within normal limits. Examination of blood (table 2) and urine revealed no abnormalities.

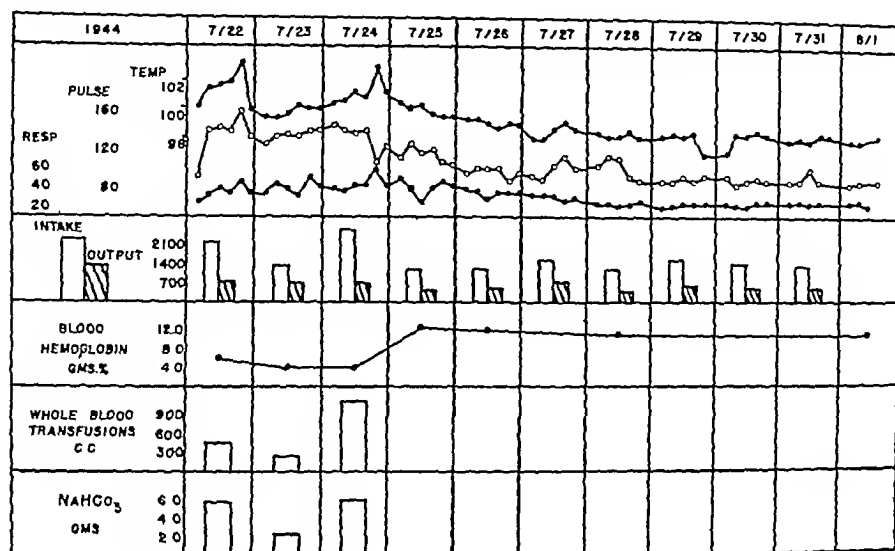


FIG 1 CLINICAL COURSE OF PATIENT WITH HEMOLYTIC ANEMIA FOLLOWING SULFADIAZINE MEDICATION

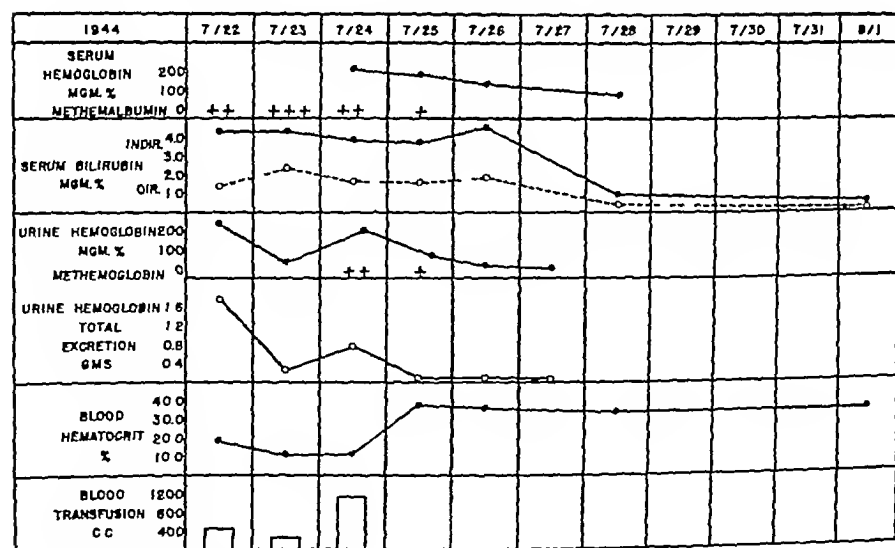


FIG 2 NORMAL AND ABNORMAL SERUM AND URINE PIGMENTS DURING THE COURSE OF THE DISEASE

October 16, 1944 Patient returned for further blood studies having been perfectly well since last seen. Physical examination was essentially negative except for enlarged tonsils. The urine was negative. Blood studies including tests for abnormal fragility of the erythrocytes and for agglutinins and hemolysins in the serum, were negative (tables 2 and 4 and fig 3).

November 2, 1944 The child was admitted to the hospital for a period of 24 hours for tonsillectomy, which was uneventful. The blood was normal.

October 8, 1945 She had been perfectly well since last seen. Development had progressed normally. Physical examination was negative. Blood studies were normal (table 2). Tests for fragility and abnormal agglutinins and hemolysins were normal (table 5).

TABLE 4—Tests for Isoagglutinins and Isohemolysins

	Patient's Cells Date				Control Cells Blood Group				
	7/22	7/23	7/24	7/26	A	B	O	AB	A B
	A H	A H	A H	A H	A	A	A	A	A
Saline	00	00	00	00	0	0	0	0	0
Patient's serum									
7/22	00	00	00	00	4+	2+	0	4+	4+
7/23	00	00	00	00					
7/24					4+	3+	0	4+	4+
7/25					4+	2+	0	4+	4+
7/26	00	00							
Group A serum	00	00	00	00	0	4+	0	4+	4+
Group B serum	00	00	00	00	4+	0	0	4+	3+
Group AB serum			±	0	0	0			

All studies performed at the following temperatures: 4° C, 25° C, 37° C

A Agglutination

H Hemolysis

TABLE 5—Tests for Abnormal Agglutinins, Hemolysins, and Fragilities

	7/22/44	7/24/44	7/26/44	9/12/44	10/16/44	10/8/45
Cold agglutinins	0	0	0	0	0	0
Cold hemolysins	0	0	0	0	0	0
Warm agglutinins	0	0	0	0	0	0
Warm hemolysins	0	0	0	0	0	0
Acid fragility		0	0	0	0	0
Hypotonic saline fragility		Increased*	Normal	Normal	Normal	Normal

All tests carried out in dilutions 1/1 through 1/256

* See Figure 3

Investigations were made to ascertain if abnormalities in the patient's erythrocytes could be produced by equilibration with human serum containing a high concentration of sulfadiazine or if the patient's serum would adversely affect normal erythrocytes containing sulfadiazine. These studies are summarized in table 6. There was no evidence that the fragility or other properties of the erythrocytes were affected by equilibration with serum containing 24 mg per cent sulfadiazine (obtained from a relatively normal group O individual who was receiving large doses of sulfadiazine) or that the patient's serum produced

abnormalities in normal erythrocytes containing a high concentration of sulfadiazine. Permission could not be obtained to study the effect of readministering sulfadiazine to the child.

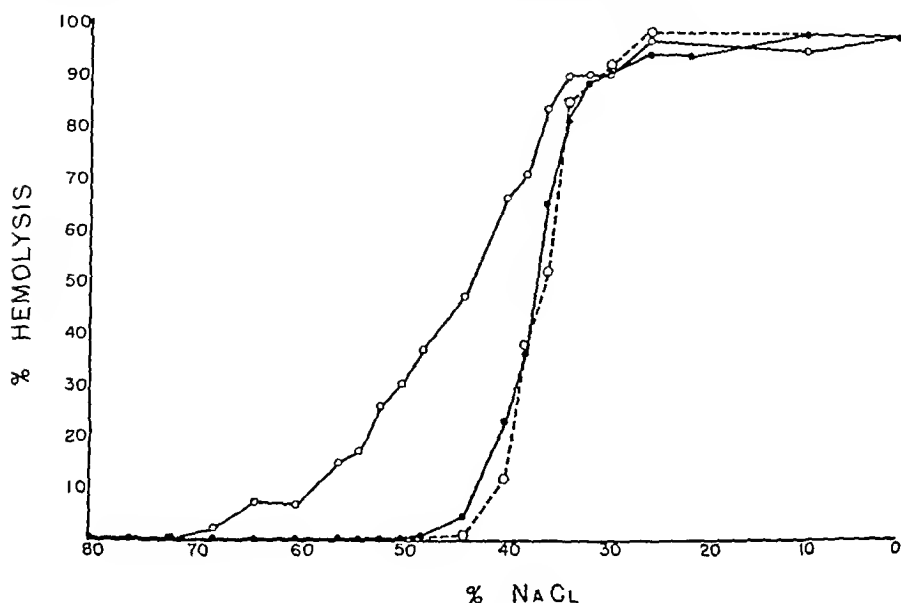


FIG. 3. FRAGILITY OF THE PATIENT'S ERYTHROCYTES IN HYPOTONIC SOLUTIONS OF SODIUM CHLORIDE

Cells obtained 7/24/44 ○—○—○
 8/1/44 ●—●—●
 10/16/44 □—□—□

TABLE 6—Effect of Serum with High Concentration of Sulfadiazine on Patient's Cells

Patient's cells equilibrated with serum containing 2.4 mg per cent of sulfadiazine (obtained from patient receiving sulfadiazine)

Cold agglutinins	Negative
Cold hemolysins	Negative
Warm agglutinins	Negative
Warm hemolysins	Negative
Hypotonic saline fragility	Normal

Effect of patient's serum on erythrocytes containing high level (2.4 mg per cent) of sulfadiazine

Cold agglutinins	Negative
Cold hemolysins	Negative
Warm agglutinins	Negative
Warm hemolysins	Negative

All tests carried out with dilutions of serum ranging from 1/1 to 1/256

DISCUSSION

It seems probable that the hemolytic process and hemoglobinuria in this patient were produced by sulfadiazine medication. No drugs other than sulfadiazine were administered during the illness prior to the development of anemia and hemo-

globinuria. There was no evidence of virus pneumonia, which is known occasionally to produce hemolytic anemia, and cold hemagglutinins were not present. No fava beans had been ingested. There was no family history of hemolytic anemia or other blood dyscrasia. The absence of acid hemolysins excluded paroxysmal nocturnal hemoglobinuria, and the negative serology for syphilis and lack of cold hemolysins ruled out cold hemoglobinuria. There were no findings to suggest that the patient had malaria and blackwater fever. Rapid recovery, subsequent good health, with completely normal blood studies over a period of more than a year, excluded congenital hemolytic jaundice and Mediterranean anemia. Moreover, after the acute phase of the illness red cell fragility tests were normal and have remained so. Repeated studies have shown no anemia, no reticulocytosis and no erythrocytic abnormalities (e.g., spherocytes, or target cells). This case had features similar to those described as idiopathic acquired hemolytic anemia, acute type. The fact that it followed and was apparently precipitated by the administration of sulfadiazine, a drug known to produce hemolytic anemia, removed it from the idiopathic group.

The acute hemolytic process appears to have been initiated in this child by only 0.75 gram of sulfadiazine, some of which was lost by vomiting shortly after ingestion. An appreciable amount was absorbed, however, since a trace of sulfadiazine was detected in the blood $22\frac{1}{2}$ hours after the last dose was administered. Intravascular hemolysis progressed so rapidly that 18 hours after the first dose of sulfadiazine had been given the concentration of hemoglobin in the plasma exceeded the renal threshold for hemoglobin and gross hemoglobinuria appeared. Twenty hours later more than half of the circulating erythrocytes had been destroyed and the patient was on the verge of vascular collapse. Although no sulfadiazine was taken after the onset of hemoglobinuria, the urine continued to contain hemoglobin for five days more. Evidences of increased blood destruction were present for two days after all sulfonamide had been cleared from the blood.

The suddenness with which hemolysis followed the ingestion of the sulfadiazine and the small quantity of the drug which was sufficient to initiate such a fulminating process suggests that the patient had become sensitized to sulfadiazine two years previously, during the two days that she took this drug. Similar instances of sensitivity to other sulfonamides have been noted previously.^{7, 8}

The nature of this sensitivity is unknown. In some way introduction of sulfonamide into the body so modifies the erythrocytes that rapid intravascular hemolysis is produced. What changes are produced in the erythrocytes and how they are brought about is of great importance in establishing the mechanism of sulfonamide hemolysis. Abnormalities in the erythrocytes have been demonstrated only in those cases of sulfonamide hemolytic anemia which were studied during the acute phase of the hemolytic process.^{2, 4, 9, 10} In these cases, as in ours, a characteristic finding has been the presence of numerous spherocytic erythrocytes and frequently a concomitant increase in the hypotonic fragility of the red blood cells. These changes may persist for only a few hours, perhaps because the abnormal cells are rapidly destroyed and replaced by new normal erythrocytes. Failure to demonstrate erythrocytic abnormality in many cases is probably due to the fact

that such cases were not studied until relatively late in the disease—that is to say, after the abnormal cells had been destroyed¹¹⁻¹⁴

Why are spherocytic cells produced in sulfonamide hemolytic anemia, and what is their relation to the mechanism of hemolysis? Ham and Castle^{10, 15} hypothesize that erythrostatics and perhaps agglutination are responsible for the production of spherocytes. They suggest that spherocytic erythrocytes are more susceptible to hemolysis because they are more readily trapped in the splenic sinusoids.¹⁵ In their published work they present no evidence that erythrostatics occurs in cases of sulfonamide hemolysis and there is no clinical evidence to support the belief that erythrostatics occurs in these cases.

Many of the reported cases of sulfonamide hemolysis have been characterized by the presence in the plasma of cold iso-hemagglutinins or autohemagglutinins.^{2, 4, 12, 16} In such cases it is quite probable that these abnormal agglutinins may account in some measure for the abnormality in the erythrocytes. However, many of these cases undoubtedly were suffering from primary atypical, or virus pneumonia, a condition which in itself produces cold hemagglutinins in high titer and which may be responsible for causing acute hemolytic anemia quite independently of any sulfonamide medication.¹⁷ It is likely that the abnormal hemagglutinins in many of these cases were produced by the primary disease rather than by the sulfonamide medication.

Dameshek² has postulated that sulfonamides may so alter some erythrocytes that they become antigenic and stimulate the development of agglutinating antibodies against the individual's own erythrocytes. This possibility may explain the development of agglutinins in those cases of sulfonamide hemolysis not associated with diseases leading to the production of hemagglutinins. However, no evidence has been presented that sulfonamides can alter erythrocytes so that they become antigenic.

In our case, careful studies demonstrated that no agglutinating or hemolyzing antibodies of any type were present in the patient's serum (table 4). Neither were the erythrocytes abnormally sensitive to isohemagglutinins (table 3). Other cases also have been reported in which spherocytosis and increased intravascular hemolysis have occurred without the appearance of agglutinins or hemolysins.¹⁸ In such cases it seems reasonable to suppose that the changes in the erythrocytes may be due to the chemical action of the sulfonamide or some abnormal metabolite of sulfonamide directly on the erythrocyte itself. Certain chemicals and toxins, e.g., phenylhydrazine and snake venom, act directly on the red cell *in vivo* and produce profound abnormalities in the structure of the erythrocyte and marked increase in susceptibility to hemolysis. It is not improbable that some sulfonamide derivative may exert an analogous action.

In our case, equilibration of the patient's erythrocytes with serum containing a very high concentration of sulfadiazine did not produce any detectable abnormality in the red cells. Although the evidence is incomplete, this suggests that some abnormal metabolite of the sulfadiazine rather than the sulfadiazine itself was responsible for the changes in the erythrocytes observed during the acute hemolytic attack. Unfortunately, studies were not made of the effect of the patient's serum

obtained during the acute hemolytic attack on the morphology and fragility characteristics of normal erythrocytes

The fact that hemolytic complications occur much more frequently following medication with certain sulfonamides (especially sulfanilamide) than with others is not incompatible with the hypothesis that an abnormal hemolytic metabolite may be responsible for the hemolysis. Certain sulfonamides may be more readily converted to a hemolytic metabolite than others. As far as is known, no hemolytic metabolite of any sulfonamide has been identified in cases of sulfonamide hemolytic anemia.

Although spherocytosis and increased susceptibility to hemolysis by hypotonic solutions of sodium chloride appear to be the only demonstrable erythrocytic abnormalities encountered in cases of sulfonamide acute hemolytic anemia, can it be assumed that these changes are responsible for the increased rate of hemolysis? The discussion of this subject is beyond the scope of this report, but it is pertinent to note that the severity of the hemolytic process in our case appeared to be correlated with the presence of large numbers of spherocytes in the peripheral blood. As the number of spherocytes decreased (perhaps because they were destroyed), the hemolytic process became less severe and finally disappeared. It has been shown that injury to red cells by various agents results in spherocytosis, which may be regarded as a pre-hemolytic phase in the destruction of erythrocytes.¹⁹

The capacity of the body to conserve the large amounts of hemoglobin liberated intravascularly in our patient was very striking. Assuming that the child's blood was normal before the onset of her illness, the total mass of hemoglobin present in her circulating erythrocytes would have been approximately 210 grams. Within a period of not longer than 36 hours, approximately 72 per cent, or 150 grams of this hemoglobin had been liberated intravascularly. Only 2.84 grams of hemoglobin escaped in the urine. The remainder was rapidly removed from the plasma, and probably was re-utilized in the formation of new hemoglobin.²⁰

The appearance of methemalbumin in the plasma is, we believe, merely a reflection of the prolonged high concentration of hemoglobin in the plasma. Methemoglobin and sulfhemoglobin were not present in either plasma or erythrocytes. Oxyhemoglobin constituted the major urinary pigment. Only small amounts of methemoglobin were present in the urine, probably because of the alkalinity of the urine and because spectroscopic examination was performed immediately after each urine specimen was voided. Methemalbumin did not appear in the urine.

The finding of erythrocytes and granular casts in the urine specimens voided shortly after admission to the hospital suggests that some degree of renal irritation had occurred. Whether or not this was produced by the sulfadiazine is uncertain, but appears improbable. The abnormality in urinary sediment rapidly cleared, and at no time was there evidence of impairment of renal function, or nitrogen retention, in spite of the marked hemoglobinemia and hemoglobinuria.

The extreme leucocytosis and the appearance of myelocytes in the peripheral blood during the period of active hemolysis were striking features. Similar leucocytic abnormalities, occasionally called leukemoid reactions, have been observed in most of the reported cases of sulfonamide hemolytic anemia. It is probable

that this leucocytic response is a reaction to intravascular hemolysis, rather than to the sulfonamide, since it is observed in various other types of intravascular hemolysis and hemoglobinemia. There was no evidence of depression or stimulation of blood platelet formation, the number of platelets remaining within normal limits.

The treatment of sulfonamide hemolytic anemia and hemoglobinuria must be directed at removing the hemolytic agent, maintaining an erythrocyte count and blood volume compatible with life, and preventing renal failure. Once the condition is suspected, all sulfonamide medication must be discontinued immediately. Diuresis should be promoted by maintaining an adequate fluid intake, by parenteral routes if necessary. The excretion of large amounts of urine accelerates the removal of sulfonamide and its metabolites from the body, and also makes the precipitation of hemoglobin derivatives in the kidney less likely. Whether or not alkalization of the urine is necessary to prevent the formation of acid hematin casts in the renal tubules is not certain, but it is safe and advisable to administer alkalinizing agents in reasonable amounts in an attempt to produce an alkaline urine. Alkalies should not be given in amounts so large as to produce disturbances in the acid-base equilibrium, since alkali medication carried to this extreme has been shown to be actually harmful.²¹

Blood transfusion is often a life-saving procedure in this disease. Hemolysis may proceed so rapidly that the erythrocyte count reaches critically low levels, and the blood volume may be so rapidly reduced that shock occurs. In our case, 1775 cc of blood were required during a period of sixty hours, a volume considerably in excess of the child's total blood volume. Only after the erythrocyte count and blood hemoglobin concentration had been restored to relatively normal levels by transfusion did the hemolytic process cease and permanent clinical improvement occur.

In many of these cases the presence of abnormal hemagglutinins in the plasma results in considerable difficulty in blood grouping and cross matching. Great care must be exercised in selecting the proper donor in such instances, and because of the existence of cold agglutinins in some cases, it may be necessary to avoid cooling the donor blood before it is introduced into the patient's vein.

An individual who has once shown a hemolytic reaction to a sulfonamide should never be given the same drug again. The hemolytic process will in all probability recur, and may be more severe than during the first attack. As demonstrated by our case, amounts of the drug so small as to be considered test doses may produce an almost fatal anemia. Whether or not a different sulfonamide compound can be given without precipitating acute hemolysis is not certain.

SUMMARY

1. A case of acute hemolytic anemia with hemoglobinuria precipitated by sulfadiazine is described.
2. Spherocytosis and increased hypotonic saline fragility were demonstrated during the acute phase of the disease.
3. Abnormal hemagglutinins and hemolysins were not present in the patient's serum.

4 The significance of these findings as related to the etiology of the disease is discussed

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PRIMARY POLYCYTHEMIA REMISSIONS INDUCED BY THERAPY WITH RADIOPHOSPHORUS

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DURING the past five years over 138 cases (cf table 2) of polycythemia vera have been treated with radiophosphorus.¹⁻⁵ There are those who believe the application of radioactive phosphorus to be superior to all other methods employed in the past, and a recent editorial⁶ contained the phrase that it may now be considered of established value. Now that the second world war is over, radiophosphorus, or P³², will be available to physicians at reasonable cost.

MATERIALS AND METHODS

Radiophosphorus* was administered intravenously as a sterile isotonic solution of sodium acid phosphate containing radioactive phosphorus. This has a half-life of 14.3 days (i.e., it loses one-half of its output of beta radiation every 14.3 days).

The average dose administered in our cases varied between 5 and 10 millicuries and was given in from 1 to 20 cc of solution, according to the concentration of radiophosphorus. Some of the cases of primary polycythemia had previously been treated elsewhere by other measures, but these patients received the same initial dose of radiophosphorus as did those with elevated red blood cell levels. Since the life span of a red blood cell is about one hundred days, the second dose of radiophosphorus, if needed, was not given until approximately three months after the first dose. Maintenance doses were given at one or two year intervals, as needed.

Standardized blood cell-counting pipets, Wintrobe hematocrit tubes, and a Klett photo-electric colorimeter were used routinely in making hematologic assays. Blood volume studies rarely gave practical information not obtained by means of hematocrit values.

The presence of secondary polycythemia (cf table 1) or pseudopolycythemia¹⁰ was ruled out in all patients. Cases of pulmonary sclerosis or fibrosis, cardiac disease, tuberculous splenitis¹ with or without splenic sclerosis,¹¹ Monge's disease,^{12, 13} or central nervous system disease¹⁴ were not treated.

RESULTS

Of the 25 cases of primary polycythemia treated with P³² that are presented in this paper (cf table 1), 17 showed satisfactory hematologic and clinical remissions,

From the Division of Hematology, Charlotte Drake Carder Foundation, Jefferson Medical College and Hospital, Philadelphia, presented in the Special Medicine Course of the postgraduate courses arranged by the American College of Physicians, Dec. 8, 1944, at Philadelphia General Hospital.

* Among other institutions at which radiophosphorus is available are: University of California, Donner Radiation Laboratory, Berkeley, Calif.; Dr. J. H. Lawrence, Massachusetts Institute of Technology, Cambridge, Mass.; Dr. Robley Evans, University of Washington, St. Louis, Mo.; Dr. Edward H. Reinhard, Carnegie Institute of Washington, Department of Terrestrial Magnetism, Washington, D. C.; Dr. D. B. Cowie.

TABLE I —Results of Radiophosphorus Therapy

Case No Sex Age at First Administration of P ³²	Previous Treatment (P = Phenylhydrazine, N = N radiation, F = Fowler's B = Bleeding) and Duration of Treatment	Before Administration of P			P ³² given intravenously		Hematologic Results				Remarks	
		Hb (%)	R B C (M)	Hematocrit (%)	Date	Amt (Mc)	Date	Hb (%)	R B C (M)	Hematocrit (%)		
PRIMARY POLYCYTHEMIA												
1 Bra F 63	P F N 7 yr	112	8 5	71	Dec 41	7 1	Apr 42	63	3 5	35	1st remission lasted 2 yr 2d remission lasted 1 yr 3d remission has lasted 6 mo so far (Jul 45) Patient states she has felt better during past 3 years than at any time in preceding 15 yr	
					Aug 42	1 5	Dec 42	69	3 4	35		
					Dec 43	1 0	Apr 43	70	4 0	37		
					Jan 44	1 5	Dec 43	104	4 9	39		
					Dec 44	5 0	Feb 44	117	6 7	38		
							Apr 44	105	5 8			
							Nov 44	104	5 5			
							Dec 44	128	6 2			
							Feb 45	106	5 1			
							Jun 45	71	3 4	38		
2 Geg F 65	P B F 5 yr	130	11 0	72	Nov 41	6 3	Dec 41	102	8 2	57	Excellent remission until family physician gave iron P ³² had to be given again excellent remission was maintained until Apr 44 when stools became tarry and W B C level dropped to 1000 in association with 5% myeloblasts and 5% normoblasts Sternal aspiration revealed many bizarre types of myeloid cells Death Apr 15 44 at age 68 Leukopenic myeloid leukemia (?)	
					Jan 43	2 0	Jun 42	80	4 7	30		
					Apr 43	4 4	Dec 42	iron administered				
					May 43	1 6	Jan 43	106	7 7	60		
					Jun 43	3 0	Mar 43	118	9 5	64		
					Jan 44	2 0	Jun 43	83	5 3	45		
							Oct 43	69	3 9			
							Feb 44	70	5 6			
							Mar 44	70	5 5			
							Apr 44	44	2 9			
3 Le G F 60	P 1 yr	162	7 8	75	Mar 43	6 1	May 43	122	9 0		Excellent remission lasting so far over 2 yr Has not missed day as schoolteacher since P ³² was administered Used to miss so many that she seriously considered giving up teaching Gained 20 lb in weight Jul 45 condition good	
					May 43	1 6	Jun 43	102	6 5	53		
					Jun 43	2 2	Sep 43	64	3 9			
							May 44	92	4 6			
							Dec 44	96	4 9			
							Mar 45	94	5 0			
							June 45	92	4 8			
4 Mac C M 64	none	149	9 5	76	Feb 43	9 3	May 43	87	5 5		1st remission lasted nearly 2 years Gained 50 lb in weight 2d remission has lasted 6 mo so far	
					May 43	2 0	Jun 43	71	3 8	35		
					Nov 44	5 0	Nov 43	87	4 2			
							Jun 44	91	4 5			
							Nov 44	118	5 6	49		
							Mar 45	87	3 3			
5 Mun F 30	P F N 2 yr	122	9 2	64	Oct 42	4 8	Jan 43	78	5 0		Excellent remission for 2½ yr so far Slight red cell elevation might be beginning of relapse (Apr 45)	
					Dec 43	1 0	Jun 43	80	4 5	40		
							Apr 44	75	5 3			
							Sep 44	76	4 1			
							Apr 45	110	5 5	54		

TABLE I—Continued

TABLE 1—Continued

Case No Sex Age at First Admin- istration of P ₂₂	Previous Treatment (P = Phenylhydrazine N = X radiation F = Fowler's B = Bleeding) and Duration of Treatment	Before Administration of P ₂₂			P ₂₂ given intravenously		Hematologic Results					Remarks
		Hb (%)	R.B.C (M)	Hematocrit (%)	Date	Amt (Mc)	Date	Hb (%)	R.B.C (M)	Hematocrit (%)		
PRIMARY POLYCYTHEMIA—Continued												
6 Mon M 54	N many courses F P 2 yr	99	8 6	54	Oct 41	7 5	Dec 41	18	4 8			Started to have a remission Returned to work Nov 41 after 2 yr layoff because of weakness and pain in abdomen Large gastric hemorrhage in 39 41 fatal one Dec 41
7 Mus M 56	none	154	11 0	74	May 42 Jan 44 Dec 45	11 0 2 0 5 0	Jun 42 Aug 42 Jan 43 Jun 43 Dec 43 Jan 44 Feb 44 Apr 44 Jun 44 Nov 44 Dec 44 Feb 45 Mar 45 Apr 45 Jun 45	135 91 82 85 91 112 118 109 95 80 134 118 95 80 83	9 1 5 9 4 1 4 4 4 5 6 6 6 8 5 4 4 2 4 6 6 5 6 6 4 6 3 6 3 9	61 41 40 59 42 51 36 38	1st remission lasted over 1½ yr 2nd remission lasted nearly 1 yr 3rd remission has lasted 6 mo so far Unable to work before P ₂₂ therapy but has not missed day since Employed as janitor	
8 Nag M 41	P N 2 yr	138	8 5	75	Apr 42 Aug 42	6 3 4 5	Aug 42 Jan 43 Jun 43 Dec 43 Jun 44 Nov 44 Jun 45	83 17 80 83 80 82 90	4 3 5 3 3 5 4 0 3 6 4 0 3 8	44 31 41	Excellent remission for over 2 yr Unable to work for 2 yr preceding P ₂₂ therapy has worked daily since	
9 Udel M 60	N B 4 yr	164	10 0	70	Jul 42	11 3	Aug 42 Oct 42 Aug 43 Dec 43 May 44 Oct 44 Nov 44 Jun 45	122 66 80 103 120 117 154 129	7 6 4 6 4 0 5 0 8 1 8 5 9 3 8 2	51 47 62 67 59	Excellent remission for 2 years then relapse Unable to work for 4 yr before P ₂₂ therapy but worked regularly until relapse Family physician has been removing blood regularly for past year (44-45) but patient has symptoms—headache dizziness weakness etc	
10 Wel F 51 (Negro)	N F 4 yr	126	11 0	76	May 42	11 5	Aug 42 Oct 42 Jul 43 Jun 44 Dec 44 Apr 45	61 66 69 60 80 75	3 4 4 5 3 7 3 4 4 1 4 0	31 35 32	Excellent remission for 3 yr so far	
11 Bach M 30	B 1 yr	164	9 1	74	Oct 43 Feb 45 Apr 45	7 5 5 0 4 0	Dec 43 Feb 44 Aug 44 Nov 44 Jan 45 Apr 45 Jun 45	131 83 99 100 134 114 95	6 7 4 1 4 5 5 7 6 8 6 8 4 8	38 45 59 56	Excellent remission for 1 yr Apparently relapse started Jan 45 2d remission started Jun 45	

TABLE I—Continued

Case No Sex Age at First Admin- istration of P ²²	Previous Treatment (P = Phenylhydrazine N = γ radiation F = Fowler's B = Bleeding) and Duration of Treatment	Before Administration of P			P ²² given intravenously		Hematologic Results						Remarks
		Hb (%)	R B C (M)	Hematocrit (%)	Date	Amt (Mc)	Date	Hb (%)	R B C (M)	Hematocrit (%)			
PRIMARY POLYCYTHEMIA—Continued													
12 Feld M 49	B 1 yr	134	7 2	60	Mar 44	4 0	Jun 44	120	6 6	53	Clinically feels much better as physician works very hard		
					Oct 44	5 0	Oct 44	101	4 9				
							Nov 44	108	5 0				
							Mar 45	108	5 9	54			
							Jun 45	110	6 0	54			
13 Gag M 59	B N P 3 yr	151	8 6	65	Apr 44	5 5	May 44	120	6 6		Unable to work as stone mason for 1 yr preceding P ²² therapy but since Aug 44 has been working daily. No hematuria (used to be very frequent) since P ²² therapy. Excellent remission for over 1 yr. Feels better than in past 1 yr.		
							Jun 44	90	4 1				
							Jul 44	60	3 1				
							Nov 44	91	3 6	46			
							Mar 45	80	4 0				
							Jun 45	78	3 6				
14 Strad M 29	B 1 yr (discharged from Sea bees when R B C level found to be 9 000 000)				Aug 44	5 5	Oct 44	99	5 1		2 mo before P ²² therapy was given had subarachnoid thrombosis. Remission of nearly 1 yr. No complaints.		
					Apr 45	5 0	Nov 44	110	5 3	54			
							Jan 45	110	5 5				
							May 45	102	5 0	50			
							Jul 45	91	4 5				
15 Sutt M 61	P daily since Apr 1930 14 yr	102	4 5	49	Sep 44	6 0	Nov 44	114	4 8	49	Feels well		
							Jan 45	93	4 5				
							May 45	95	4 5				
							Aug 45	83	4 3	44			
16 Mor F 30	none	160	10 0	77	Aug 44	5 0	Oct 44	99	5 6		Married 5 yr but had no pregnancy as desired. About 1 mo after P ²² therapy patient became pregnant. Delivered of 5 lb 8 oz still born child normally formed with twisted cord. Apr 29 45 Jun 45 menstruating normally. Aug 45 pregnant again.		
							Nov 44	79	4 9	38			
							Jan 45	54	2 7	22			
							Apr 45	73	3 7	36			
							Jun 45	75	4 3	35			
							Aug 45	75	4 4				
17 Man F 42	B 1 yr	170	8 4	61	Sep 44	4 0	Nov 44	85	4 4	45	Excellent remission for nearly 1 yr		
							Feb 45	68	3 7	35			
							May 45	67	3 9				
							Jun 45	87	4 1	39			
18 Sit M 57	P N 6-7 yr B 3 yr	103	8 6	60	Oct 44	5 0	Dec 44	110	8 0	60	Rather refractory to P ²² . Has hypertension.		
					Dec 44	5 0	Mar 45	106	6 0	58			
					Feb 45	5 0	May 45	95	5 3	53			
					Jun 45	5 0	Jun 45	104	6 1				
							Aug 45	90	4 7				

TABLE 1—*Concluded*

TABLE 1—Continued

Case No Sex Age at First Admin- istration of P ₃₂	Previous Treatment P = Phenylhydrazine X = X-ray radiation F = Fowler's B = Bleeding and Duration of Treatment	Before Administration of P ₃₂			Previously given intravenously		Hematologic Results					Remarks
		Hb (%)	R B C (M)	Hematocrit (%)	Date	Amt (Mc)	Date	Hb (%)	R B C (M)	Hematocrit (%)		

PRIMARY POLYCYTHEMIA—Continued

19 Kauf F 36	P X 7 yr	130	8 6	64	Oct 44	7 5	Feb 45 Jun 45	11 11	3 1 3 3	35	Frequently has had as much as 1500 cc of blood removed at one time during past 5 yr June 45 states she has never felt better
20 Fran M 50	F B 2 yr	93	5 8 after phlebotomy	46	Nov 44	5 0	Feb 45 Jun 45	100 90	6 2 3 9	52 42	Excellent remission
21 Hech M 50	X 1 yr B 6 yr (irreg)	144	7 0	68	Nov 44 Mar 45	5 0 5 0	Mar 45 May 45	126 129	1 3 8 5	65 68	Refractory We believe patient has early myeloid leukemia
22 Rage F 49	none	134	7 2	64	Apr 45	5 0	May 45 Jun 45 Aug 45	114 100 100	6 2 6 7 5 5	61	Remission just starting
23 Bake M 67	B 5 yr	90	5 7	51	Jun 45	5 0	Aug 45	18	4 8		Treatment just started
24 Avor M 63	B 6 yr	124	8 4		Aug 45	5 0					Treatment just started
25 Hack F 68	P 4 mo	131	8 3		Aug 45	5 0					Treatment just started

SECONDARY POLYCYTHEMIA

1 Schw F 36	B 1 yr	153	8 1		May 44 Nov 44	8 5 5 0	Jul 44 Nov 44 Jun 45	114 128 120	5 3 5 9 5 5	56 61 60	Heart disease decompensation
2 Bree F 35	mitral stenosis 2 yr	110	6 0 (Jun 44)	55			Jun 45	85	5 1	41	Well compensated during past year digitalis and rest 1% normoblasts noted in otherwise normal differential
3 Sink F 62	intracellar tumor	144	7 3 (Jun 44)	60			Jun 45	90	6 2		X-ray therapy to pituitary area with marked improvement during year
4 Wasel M 50	pulmonary fibrosis cardiac decompensation	128	5 9	66							Death 6 mo later cardiac decompensation
5 Poll M 20	hypoplasia of aorta	136	8 5	60							Continues to have cyanotic coughs up blood occasionally

3 showed unsatisfactory remissions, and in 3 the therapy was of too recent date to warrant drawing of conclusions. The duration of the remissions was from six months to three years. One patient died after a huge gastric hemorrhage, and another after developing leukopenic myeloid leukemia.

COMMENT

In table 2 are listed a few of the hematologic clinics using radiophosphorus for treatment of polycythemia, together with the results they have obtained.

Of the five deaths reported (cf. table 3), three were due to leukopenic myeloid leukemia. Leukopenic myeloid leukemia as a terminal event in polycythemic pa-

TABLE 2.—*Collected Results in the Treatment of Polycythemia Vera with Radiophosphorus*

Hematologic Clinic	No. Cases	No Satisfactory Remissions	Unsatisfactory Remissions	No. of Deaths	Causes of Death
Jefferson	22	17	3	1	hemorrhage
				1	leukemia
Mayo	38	36	2	1	leukemia
Univ. Calif. ⁵	50	47	3	1	leukemia
				1	hypernephroma
Univ. Penn. ⁴	8	7	1		
Mr. Sinai (New York) ³	14	12	2		
New England Deaconess (Boston) ³	3	2	1		
Totals	138	124	11	5	

TABLE 3.—*Deaths Following the Use of Radiophosphorus*

Hematologic Clinic	Cause of Death	Age at Death	Duration of Polycythemia before P ³² Treatment (yr.)	Total P ³² Dosage (Mc.)	No. between Last P ³² Dose and Death
Jefferson	gastric hemorrhage	54	2	7.5	2
	leukemia	68	5	19.3	4
Mayo	leukemia	63	3½	7.0	16
Univ. Calif.	leukemia	69	8	44.0	20
	hypernephroma	64	2	41.5	26

tients receiving spray X radiation has been reported.¹⁵ Tinney et al.¹⁶ reported that among 163 cases of polycythemia, 17 patients (10 per cent) died of leukemia. They did not state how many of the 17 cases were of the leukopenic type. None of the 163 cases had been treated with radioactive phosphorus.

The duration of remissions (from six months to five years) following the use of radiophosphorus is comparable to the duration of remissions following spray X radiation.^{17,18} A remission can be stopped almost immediately if iron or large amounts of liver or red meat are given (cf. case 2, table 1). Therefore all patients with polycythemia were placed on a red meat-free diet. Many cases with unusually long remissions are reported.¹⁹⁻²²

Table 1 includes the records of 5 cases* of secondary polycythemia, in the first of which radiophosphorus was given on two occasions purely as a therapeutic trial. As was expected, no reduction of the red cell level occurred in this case of cardiac decompensation. This is additional proof that radiophosphorus should be given only in cases of primary polycythemia.

CONCLUSION

Intravenous injection of radiophosphorus induces satisfactory remissions in most cases of primary polycythemia (polycythemia vera).

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* In one of these cases, Dr. F. B. Wagner, by injecting radio-opaque substances into the right and left auricles of the heart through ureteral catheters inserted into the basilic vein and brachial artery respectively, was able to establish the diagnosis of hypoplasia of the aorta and secondary dilatation of the pulmonary artery.²³

PROGNOSIS IN THE NEUROLOGIC MANIFESTATIONS OF PERNICIOUS ANEMIA

By R. WAYNE RUNDLES, PH D, M D

FOLLOWING the introduction of liver therapy for pernicious anemia in 1926¹⁹ the curability of the neurologic manifestations of this disease became a matter of intense interest and large series of patients with acute, chronic and residual neurologic defects were treated with lightly cooked liver and oral liver extracts. It was found that while this treatment restored satisfactory blood levels and reversed the atrophic and inflammatory lingual changes the neurologic benefit was far less conspicuous. Some investigators concluded that liver therapy resulted in no neurologic improvement beyond that due to the correction of the anemia and betterment of the patient's general condition, and that progressive disability might occur in some cases in spite of treatment.^{10 12} The most optimistic observers were of the opinion that adequate treatment arrested the progression of the disease and that some degree of functional improvement might occur when treatment was begun reasonably early.^{1 8 16 18 19 25 28 31}

In recent years the general outlook regarding the neurologic prognosis in pernicious anemia has been altered by several factors. For one, the diagnosis is now made earlier in the illness and the neurologic disease is less advanced when treatment is begun. Concentrated liver extracts for parenteral use, although refined and tested only to give the maximal hematologic response, have abolished the uncertainties of oral types of therapy, and optimal treatment is now possible in all cases. The demonstration of unequivocal degenerative changes in peripheral nerves removed by biopsy^{9 11 28} has finally substantiated the many suggestions that peripheral neuropathy rather than spinal cord degeneration is actually responsible for many of the typical neurologic signs and symptoms.^{1 7 13 18 29 31 32} The great recuperative power of the peripheral nerves indicates, of course, a far greater potential curability. Finally, the isolation of new vitamins in the B group has permitted experiments bearing on the problems of diagnosis and therapy. While the common crystalline vitamin B components have failed to be of direct value in the treatment of any of the clinical manifestations of pernicious anemia, each new one isolated has to be evaluated as a possible substitute or adjunct to standard therapy.

The present report is based on a study of 20 recent patients with the neurologic manifestations of pernicious anemia whom we were able to follow with frequently repeated examinations from the start of treatment of pernicious anemia in relapse until maximum neurologic recovery had occurred. Somewhat less than one half of all our new patients were judged to have significant neurologic disease. Those chosen for study appeared to be typical of the large number of patients with this

From the Department of Medicine, Duke University School of Medicine. This study was begun at the Simpson Memorial Institute, University of Michigan. The crystalline vitamin products were furnished by the Upjohn Company.

disease treated and followed at the Simpson Memorial Institute over a period of years.¹⁰ It will be apparent from the discussion to follow that the neurologic manifestations of pernicious anemia should be carefully evaluated both for diagnosis and for prognosis and not merely receive the banal designation posterolateral sclerosis or cord degeneration. Four types of disturbed neurologic function actually occur in pernicious anemia: (1) cerebral, (2) olfactory, (3) peripheral nerve and posterior columns of the spinal cord, and (4) lateral columns of the spinal cord. The incidence of the different types of neurologic disturbance in the group of patients studied is given in Table 1.

1 *Cerebral Symptoms* The cerebral symptoms of pernicious anemia^{3, 10, 18, 21} are those of the so-called organic brain syndrome, loss of memory, diminished intellectual capacity, confusion, etc. Psychoses with delusions and hallucinations may occur but the symptoms often suggest senility, except perhaps for an unduly rapid onset. The appearance of these symptoms in combination with other mani-

TABLE 1—Types of Neurologic Dysfunction in 20 Patients with Pernicious Anemia

Cerebral	3 cases
Olfactory	4 cases
Peripheral nerve and posterior columns of the spinal cord	11 cases
Peripheral nerve, posterior and lateral columns of the spinal cord	9 cases

festations of pernicious anemia and their response to treatment are illustrated by the following 2 case histories.

S. D., a 61-year-old telegraph linesman, had been healthy and worked regularly until 4 months before he was seen, when pronounced loss of memory, confusion, and slow difficult speech became apparent. A few weeks later numbness and tingling of his feet and legs as high as the knees and of his hands developed. His gait became unsteady and he was forced to quit work. His general condition became progressively worse and finally he could walk only with the support of others. Physical examination showed a cooperative but confused male, well developed and well nourished. His tongue was not coated but was normal in color and showed only slight flattening of the papillae at the lingual margins. He was unable to walk except by holding on to the furniture. In the Romberg position standing with the heels together and eyes closed he could not stand without falling. The tendon reflexes were normally and symmetrically active except for the ankle jerks which were absent. The vibratory sense was diminished at the pelvis, absent at the knees and below. Motion and position sensibilities were greatly impaired in the lower legs. The plantar response was usually extensor.

He was first seen by a neurologist who insisted that the diagnosis was senility and then by an internist whose major diagnosis was generalized arteriosclerosis. He was referred for a blood examination which showed the RBC to be 3.3 million, WBC 8,000, hemoglobin 13.1 Gm per 100 cc, hematocrit 38 per cent, and the mean cell volume 115 cu microns. The appearance of the stained cells and the differential white cell count were not definitely abnormal. A presumptive diagnosis of pernicious anemia was made and he was started on intramuscular injections of purified liver extract. In one month's time the RBC had risen to 4.4 million, the hemoglobin to 14.5 Gm, and the macrocytosis had disappeared. The diagnosis of pernicious anemia was considered as established. After 7 weeks of treatment the patient believed that slight neurologic improvement had occurred but the neurologic examination showed little change.

He was examined again at the end of 5 months of treatment when he appeared definitely more alert and no longer confused. His family had noted the improvement in his mental status also and thought that he was again completely normal. He was able to walk steadily and with rapid progression without support, and in the Romberg position he could stand with only moderate swaying. The tendon reflexes

were normal. The perception of vibration had returned to normal at the pelvis, but was diminished at the knees and absent at the ankles. Motion and position senses in the lower legs were not demonstrably impaired. The Babinski signs were still inconstantly present. One year after the start of treatment he was able to return to work as a telegraph linesman.

L. M., a 54 year old minister, had enjoyed good health until about one year previously when he began to faint occasionally in the pulpit while preaching and people began to note that his sermons were rambling and less coherent. His memory deteriorated and on several occasions he awakened his wife in the middle of the night to be reassured that he had attended meetings and performed marriage ceremonies that he had promised. He became absent minded, ignored traffic lights, and was given several police summons for speeding. His family physician advised him to obtain a number of diagnostic studies at a local hospital and later several psychiatric interviews but these led to no recommendations for treatment. Two to three months after the onset of the mental symptoms numbness and tingling appeared in his extremities and in a few more weeks his gait became staggering and somewhat slapping. Word circulated in the community that he was suffering from an incurable and progressive disease, presumably tabes dorsalis, and the moral virtue of his first wife was questioned. It became impossible for him to officiate at some of the church services such as communion without stumbling and falling and his bishop advised him to take several weeks vacation. The rest was of no benefit and he was then given a permanent retirement with pension. His disability progressed until one week before his hospital admission he became entirely unable to walk. He was very confused and sometimes suspicious and antagonistic. On the day before admission to the hospital urinary retention developed. It was later learned that he had had the abrupt onset of complete sexual impotence early in his illness.

Examination on admission showed that he was well developed and well nourished. He was cooperative but appeared markedly depressed and emotionally labile. He could converse with good articulation and vocabulary although he was moderately disoriented, often confused and incoherent. There was no lingual abnormality. His bladder was palpable midway to the umbilicus. On neurologic examination he was unable to stand without holding on to stationary objects. He could move his legs only uncertainly and with visual guidance. The tendon reflexes were absent at the knees and ankles. The cutaneous sensibilities were blunted over both feet. The vibratory sense was diminished at the wrists, absent at the pelvis and below. Motion and position senses were absent in the lower extremities and on heel to shin testing there was a mild ataxia. There was no ankle clonus. The plantar responses were extensor.

The blood examination showed the red cell count to be 3.9 million, hemoglobin 15.7 Gm. per 100 cc, WBC 5,050, hematocrit 46 per cent and the average cell volume 118 cu. microns. The morphology of the red cells in the stained films and the differential white cell count were not abnormal. There was no free hydrochloric acid in the gastric contents after the subcutaneous injection of histamine. Both the blood and spinal fluid gave negative serologic tests for syphilis.

On two occasions during the first few days in the hospital urinary retention necessitated catheterization. Two weeks later when the only urinary symptoms were hesitancy and a weak stream a cystometric examination showed that there was an atonic bladder paralysis with the bladder capacity over 700 cc, sense of filling and first desire to void at 550 cc, residual urine 120 cc and a very low intravesical pressure.

For 5 days while diagnostic studies were being completed he was given daily intramuscular injections of a mixture of crystalline vitamin B components containing 50 mg. of thiamine chloride, 10 mg. riboflavin, 5 mg. pyridoxine, 50 mg. calcium pantothenate and 250 mg. nicotinamide. His mental symptoms and neurologic status remained unchanged. He was then started on injections of purified liver extract, 15 units daily which after discharge home was reduced to 30 units per week. He soon became stronger and after 3 weeks of treatment he was able to walk about the ward holding on to furniture. He returned home and in 2 months time was able to take a daily walk of 2 miles using a cane.

He was examined again at the end of 5 months treatment. A tendency toward depression remained but his intellectual interests had returned and he was spending much of his time reading and composing sermons. He was able to walk with rapid progression without conspicuous unsteadiness. He was able to stand in the Romberg position with only slight swaying. The patellar reflexes had returned but the achilles reflexes were still absent. There was no impairment of the cutaneous sensibilities. The heel to knee test was well performed. The vibratory sense was absent at the pelvis and below and the plantar

responses were inconstantly extensor. There were no urinary complaints and a cystometric examination was entirely normal except for a slightly delayed sense of fill and a somewhat low intravesical pressure.

One year after the beginning of treatment he and his wife believed that his intellectual ability and interests had completely returned to normal. He had not resumed work apparently because of residual doubts concerning the nature of his illness in the minds of his parishioners. Examination showed that his gait was still slightly unsteady and there was some swaying in the Romberg position. Both the patellar and achilles reflexes had become moderately hyperactive. There was no ankle clonus. The vibratory sense was still absent at the pelvis and below and the plantar responses were still inconstantly extensor.

2. *Olfactory Symptoms* A second type of neurologic disturbance occurring in patients with pernicious anemia involves the olfactory sense.²² While occasionally a nearly complete anosmia develops, the commonest symptom is perversion or loss of taste for food, especially for protein foods such as meat and eggs. This alteration occurs independently of atrophic and inflammatory changes of the lingual mucosa. A typical case history follows.

J. D. R., a 55 year old physician, had noted for some 6 months anorexia, undue fatigue and showers of tingling in his extremities. He was accustomed to having for lunch a chicken salad sandwich at a hotel noted for the excellence of its cuisine. The sandwiches began to taste putrid to him and he sent a number back to the kitchen before he became aware that other people continued to eat them with enjoyment. Soon antiseptics and other chemicals in his office lost their characteristic odors and often smelled like coffee. In a few more weeks numbness, tingling and cold feelings were continually present in his hands and feet. After sundry speculative diagnoses had been offered a red blood cell count of 3.8 million with a macrocytosis of 106 cu. microns and gastric achlorhydria following the injection of histamine were discovered. The appearance of his tongue was not definitely abnormal. The only objective neurologic abnormality concerned the vibratory sense which was diminished at the knees and absent at the ankles. After one month of intramuscular liver therapy the blood values were normal, his appetite was excellent, his olfactory symptoms had disappeared and his vibratory sense was no longer impaired.

3. *Peripheral Nerve and Posterior Column Disease* The commonest and in most cases the earliest type of neurologic disorder in patients with pernicious anemia we now attribute to involvement first of the peripheral nerves and secondarily of the posterior columns of the spinal cord.^{1, 7, 9, 11, 13, 18, 28, 29, 31, 3} The symptoms and signs often mimic exactly those of neuritic diseases of different etiology and include muscle weakness with tender and later atrophic muscles, and paresthesias such as numbness, tingling, cold feelings, etc., with impairment of the cutaneous sensibilities distally in the extremities. A characteristic finding of the greatest value in differentiating the neurologic abnormalities of pernicious anemia from those of other diseases is the early and severe impairment of the sensibilities of vibration, motion and position. These sensibilities are mediated by the fiber components of the peripheral nerves whose central processes constitute the posterior columns of the spinal cord. The selective involvement of these neurons has been widely interpreted as indicating a primary posterior column degeneration but this idea has never been supported with convincing pathologic studies of both peripheral nerves and spinal cord. There is obviously no clinical means by which impairment of the vibratory, motion and position senses can be distinguished as occurring either peripherally or in the posterior columns of the spinal cord, since but one neuron is involved, except perhaps by the study of the prognosis in different degrees of disease after effective treatment has been instituted.

After neuritic symptoms have appeared distally in the upper extremities there is little tendency for extension above the wrists and forearms. In the lower extremities, however, the disturbance ascends sometimes acutely to the level of the mid-abdomen, or rarely to the level of the mid-thorax, and may simulate a transverse or segmental lesion of the spinal cord. Coincident with this progression disturbed gastro-intestinal or genito-urinary function and in about half of the cases evidence of disease of the lateral columns of the spinal cord develop.

4 *Lateral Column Disease* Involvement of the lateral columns of the spinal cord in patients with pernicious anemia represents the final stage in the progression of the neurologic disease and rarely, if ever, appears without adequate warning by the less serious peripheral nerve symptoms beforehand. Typical clinical evidence of simple lateral column disease includes the complaints of spastic movements, stiffness and cramping of the muscles, and the finding of exaggerated tendon reflexes, clonus and extensor plantar reflexes. The accompanying peripheral nerve disease in pernicious anemia, however, often modifies this picture to produce a flaccid paralysis with loss of tendon reflexes in spite of signs of lateral column involvement.^{12 22 28 29} During the course of treatment as peripheral nerve function is restored the tendon reflexes reappear and may even become exaggerated with clonus while the abnormal plantar responses persist. The interpretation of this sequence as representing progression of the neurologic disorder is in error.

5 *Intestinal and Bladder Disturbances* A wide variety of gastro-intestinal symptoms are encountered in patients with pernicious anemia and among these is a group that appears to be related to neurologic dysfunction. To be excluded are such common chronic complaints as vague indigestion, gaseous indigestion, a tendency toward frequent soft stools, etc., which persist without change in uncomplicated cases during times of spontaneous or therapeutic remission. Again when the hemoglobin level falls below about 5 Gm per 100 cc most patients develop anorexia with vomiting. They become able to eat again when the anemia is alleviated by blood transfusion. Gastric retention has been demonstrated by roentgen study in such cases.¹⁶ Many patients with no more than a moderately severe anemia develop during relapse of their disease a profound distaste for food, lack of appetite, a post-prandial feeling of distressing fullness, etc., which disappears 2 to 4 days after the beginning of treatment before other notable response to treatment occurs. The nature of this functional change is obscure.

The gastro-intestinal symptoms which appear to be of neurologic origin occur characteristically in patients with the more severe degrees of neuritic disorder, especially in those with neurogenic bladder paralysis. The earliest and most persistent symptom is severe constipation often requiring the continual use of enemas or massive catharsis. Subsequently post-prandial abdominal distention, cramps, borborygmi and, in long untreated cases, diarrhea with sphincter weakness develop. In these patients roentgen study has revealed grossly disturbed small intestinal function with variable caliber, altered speed of transit, and loss of normal mucosal pattern.¹⁴ The symptoms do not subside until after several weeks or months of treatment have been given and neurologic improvement has resulted. With the improvement in the gastro-intestinal symptoms, although an increased tendency

toward constipation may persist indefinitely, there is also a return to normal in the roentgen appearance of the intestinal tract

Disturbed genito-urinary function is a well known late result of neurologic disease in patients with pernicious anemia but the details of the functional deficit as related to the general neurologic condition as well as the eventual prognosis have received very little study. The earliest symptom to appear in male patients is usually impotence. Hesitancy, weakness of the urinary stream, and finally urinary retention, dribbling, or overflow incontinence develop later. Most present day patients do not allow incontinence or obstructive urinary symptoms to persist for more than a short time before seeking adequate treatment. Neurologic examination in these patients invariably shows cutaneous sensory impairment in the lower legs and diminished or absent vibratory sense to the level of the iliac crests. Evidence of lateral column disease may or may not be present. Cystometric examination usually discloses the presence of an atonic bladder paralysis with impaired sense of bladder filling, very low intravesical pressure, increased bladder capacity, and variable amounts of residual urine. Mechanical obstructive lesions must be ruled out before a neurogenic etiology can be certain. In some patients with disease of the lateral columns of the spinal cord uninhibited contractions may be recorded on the cystometric curve and the bladder capacity, tone and functional behavior found to be more variable. Too few patients of this type have been carefully evaluated and followed for an accurate prognosis to be possible under all circumstances. Most observers agree that bladder neck and sphincter symptoms may disappear with treatment of the pernicious anemia.^{8 15 27} Three of our recent patients, one of them a woman, with obstructive urinary symptoms and atonic bladder paralysis of fairly short duration regained normal bladder function as regards both symptoms and cystometric findings after a few weeks or months of liver therapy. The prognosis when the paralysis is of longer duration or when there is severe lateral column disease is undoubtedly less favorable. Urologic measures designed to avoid mechanical damage to the detrusor muscle, urinary tract infection, and bladder neck obstruction are of prime importance during the period of recovery.

Therapy Uncertainties regarding the possible role of primary or conditioned vitamin B deficiencies in the etiology of the neurologic manifestations of pernicious anemia have often led to the recommendation that crude liver extracts and sometimes vitamin supplements be used in treatment.^{1 7 31} Recent assays of the vitamin content of various liver extracts have shown, however, that the less refined products are not actually richer proportionately in known vitamin B components and that the absolute amounts present in any case are now of little therapeutic importance.⁶ It has been found furthermore that the purified vitamin B fractions have no curative effect on the lingual changes of pernicious anemia and that, with the exception of folic acid, they do not possess antianemic properties in this or in the related macrocytic anemias.^{17 20 24 30 31} Castle and his collaborators⁵ conducted experiments in which purified casein, normal human gastric juice, and a mixture of 11 of the isolated members of the vitamin B group were fed simultaneously to patients with pernicious anemia in relapse. No hematologic response was observed.

To investigate the effects of the parenteral administration of some of the crystalline vitamin B substances particularly on the neurologic manifestations of pernicious anemia, 5 suitable patients were given a mixture containing 10-60 mg of thiamine chloride, 10 mg of riboflavin, 5 mg of pyridoxine, 50 mg of calcium pantothenate, and 250 mg of nicotinamide, intramuscularly every day over periods of 7 to 10 days. One patient was given 450 mg of nicotinamide daily by mouth in addition and another patient was given a yeast concentrate containing folic acid during a separate trial period. In no case was there any observable hematologic, lingual, or neurologic improvement. After vitamin B therapy was discontinued a definite response was obtained within a similar period of time with purified liver extract, which contains negligible amounts of the same vitamins. The administration of the vitamin B products before the liver did not appear to accelerate or modify this response in any manner. The observations in one of these cases were as follows:

F. B., a 44 year old printer, had been entirely well until one year previously when he began to note numbness and tingling in his fingers. The paresthesias extended and for 6 to 8 months had been felt in his hands, lower extremities and trunk as high as his waist. For 4 to 5 months all types of food had tasted the same and he acquired a dislike for meat. At times his tongue was sore and noticeably red. He became severely constipated and his appetite vanished. Some hesitancy on urination and complete impotence developed. Over a period of 3 months he took several hundred capsules containing a vitamin B mixture but he steadily became worse. Two months before his hospital admission his gait became staggering and for 2 to 3 weeks he had been unable to walk at all without being supported.

Physical examination showed a well developed and fairly well nourished white male. There was a faintly yellowish cast to his skin. His tongue was pinkish lemon in color and was entirely devoid of papillae. When standing up he reeled about and could not stand or walk without support on both sides. The cutaneous senses were blunted over the hands and below the knees. There was dependent rubor of the feet which vanished quickly with elevation. The vibratory sense was diminished at the pelvis and nearly absent at the knees and ankles. The heel to shin test was very poorly performed and the motion and position senses were moderately impaired. The tendon reflexes in the upper extremities were normally active but those at the ankles and knees were exaggerated. Sustained ankle clonus and Babinski signs were present bilaterally.

Examination of the peripheral blood showed the red blood cell count to be 2.2 million, WBC 2800, hemoglobin 7.9 Gm per 100 cc, hematocrit 28 per cent and average cell volume 127 cu microns. In the stained blood films the erythrocytes varied greatly in size and in shape, reticulocytes were slightly less than 1 per cent, and the percentage of neutrophils was reduced with many of them having multilobed nuclei. The number of platelets was diminished. A gastric analysis with histamine showed gastric achylia. Roentgen examination of the gastro-intestinal tract revealed no abnormality except a functional disturbance of the small intestine. A cystometric examination was not definitely abnormal.

For experimental purposes he was given over a period of 7 days a yeast concentrate containing folic acid. Daily reticulocyte counts were made and there was no hematologic response, his tongue showed no improvement, the anorexia persisted and his neurologic status remained unchanged. During a second period of 7 days he was given every day by injection 10 mg of thiamine chloride, 10 mg of riboflavin, 5 mg of pyridoxine, 50 mg of calcium pantothenate, and 250 mg of nicotinamide. At the end of the second 7 day period there was again no therapeutic response in any regard. The vitamin B therapy was discontinued and daily intramuscular injections of 15 units of purified liver extract begun. At the end of the first week of liver therapy the expected reticulocyte response had occurred, his appetite had improved, and there was visible regeneration of the lingual papillae. The patient thought that there was some improvement neurologically as regards muscular strength and the paresthesias. At the end of 2 weeks the neurologic improvement was undoubted since he was able to walk about holding on to furniture.

At home he continued to take injections of 30 to 45 units of liver extract each week. At the end of 5

months he had returned to full time work. Minor paresthesias persisted about his fingers and toes. His blood values were found to be entirely normal. Neurologic examination showed that he was able to walk with good speed and strength although his gait was somewhat unsteady and stiff. When standing in the Romberg position there was a moderate amount of unsteadiness. The only demonstrable sensory impairment was diminished vibratory sense over the toes. The knee and ankle reflexes were exaggerated but there was no ankle clonus and the plantar responses were flexor. During the next 3 months there was still further neurologic improvement after which his condition remained stable. At that time his appetite, taste for food and digestion were again completely normal and only a tendency toward constipation remained. There were no urinary symptoms and his normal sexual potency had returned. Although his legs felt stiff and he found it difficult to walk rapidly his usual gait appeared nearly normal. There was minimal swaying when he stood in the Romberg position. The remainder of his neurologic examination was as before and he regarded the neurologic residuals as being inconsequential.

In view of the lack of demonstrable therapeutic response of either the lingual, anemic, or neurologic manifestations of pernicious anemia to the administration of the common vitamin B components, and the fact that all these manifestations do respond more or less in parallel with liver therapy, the emphasis in the treatment of those with neurologic disability should certainly be placed on giving the optimal amount of the antianemic liver principle. Although quantitative estimations are obviously difficult in this regard, it seems to be a matter of universal experience that larger amounts of liver should be used in the treatment of those with neurologic complications than are required for a maximal hematologic response. Parenteral therapy is always advisable in these cases. Our standard treatment regimen has been the injection of 30 to 60 units of concentrated liver extract a week until there has been a maximum neurologic recovery. When this has occurred a permanent remission with complete assurance that neurologic relapse will not occur is obtained by the regular injection of about 15 units every 10 to 14 days. We have preferred to use the concentrated liver extracts because of the ease with which the comparatively large unitage may be given and to avoid the discomfort and the danger of inadequate dosage attending the use of the crude extracts. Most of our recent patients have, also, been treated without supplementary yeast or vitamin B products and the rate and degree of neurologic recovery appears to be as satisfactory as when these have been added.

Prognosis. It has become evident from following the course of recovery of patients with different types and degrees of neurologic disease that a relatively accurate prognosis can generally be given at the start of treatment. As indicated in the individual case histories cited, the over-all improvement in the neurologic status is far greater than generally recognized. Even patients unable to stand or those with nearly complete loss of voluntary control of the lower extremities, especially when this is of acute onset and less than one or two months in duration, may recover to such a degree that only minimal neurologic abnormalities can be detected later.²⁷⁻²⁸ The cerebral or mental symptoms which occur as a part of the neurologic disorder in patients with pernicious anemia appear to be completely curable although 6 to 12 months may be required to accomplish this. Perversion of the olfactory sense and the partial anosmia have likewise been completely cured by liver therapy in from 2 to 4 months in all the patients we have been able to follow.

In the peripheral nerve and associated posterior column disease the degree of recovery depends upon the qualitative defect present, its severity and its duration.^{8 15} The earliest sign of neurologic improvement is the disappearance of muscle tenderness, when this is present, and an increase in muscular strength and motor performance which became apparent as early as one to 2 weeks after the start of treatment. Even muscular weakness and atrophy severe enough to confine patients to bed responds to treatment within a few weeks in those who are not bedridden or severely crippled for other reasons and there is no residual defect. Impairment of the superficial sensibilities with paresthesias of a few weeks or months duration in which cutaneous anesthesia has not resulted can be expected to clear without residual symptoms other than perhaps slight tingling distally. When complete loss of cutaneous sensibilities has occurred distally in the extremities some degree of permanent sensory blunting with troublesome paresthesias, especially that of tingling, is usual. Of the sensibilities mediated by the posterior columns the vibratory sense, being affected first and most severely, is most likely to be permanently impaired. When the perception of vibration is not completely lost at a given bony prominence a return to normal may be predicted. When a complete loss as high as the pelvis has occurred a permanent defect at least at the level of the knees and below will remain, although this is of little consequence to the individual concerned. In patients with ataxic symptoms up to several weeks in duration the senses of motion and of position will usually improve with treatment until there are no residual signs except perhaps for some unsteadiness when the patient stands in the Romberg position. The loss of tendon reflexes, except at the ankles, is seldom if ever permanent and as they return in patients with lateral column disease exaggerated reflexes with clonus may develop as the end result. Neurologic defects resulting from disease of the lateral columns of the spinal cord carry the poorest prognosis of all. It is not rare, however, for extensor plantar reflexes presumably of short duration to revert to normal after treatment is begun. When symptoms of lateral column disease have been present for longer than one to 2 months the abnormal plantar responses and exaggerated tendon reflexes will almost invariably persist permanently. The functional defect as far as the motor ability of the patient is concerned is not necessarily of great consequence. With vigorous therapy using potent liver extracts the neurologic improvement in pernicious anemia is most rapid after 4 to 6 weeks of treatment and becomes slower after 4 to 6 months. Little if any improvement in the neurologic status can be anticipated after 10 to 12 months. When the response to liver extract is used as a diagnostic test in neurologic disorders of uncertain relationship to pernicious anemia there is no reason to prolong the trial of liver therapy beyond the 4 to 6 month period if definite improvement has not resulted.

The neurologic residuals which remain after a period of intensive treatment as outlined represent degenerative changes for which an effective type of therapy can in all probability never be developed. Prevention of neurologic disability thus depends to an important degree upon the early and accurate diagnosis of pernicious anemia. This unfortunately seems to be a difficult matter for most physicians. More emphasis should be placed on the clinical extremes of the disease, the ob-

scure and the atypical cases,²⁻²² and less on anthropologic characteristics of little practical diagnostic significance and on the so-called average or typical features formerly observed in patients succumbing to the disease after several relapses in the days before effective therapy was available.⁴ The lingual, anemic, and neurologic manifestations occur largely independently of each other, and even in individual patients undergoing successive relapses of the disease lingual or anemic changes may predominate on one occasion and neurologic disease on another.^{9, 10, 27, 31} An early neurologic diagnosis is often possible in patients whose complaints and findings suggest only peripheral neuropathy or organic cerebral changes. Serious neurologic disease may develop before there is notable deterioration of the blood values²⁶ and in these cases the finding of a slightly decreased red cell count with macrocytosis, often overlooked in the work of a routine laboratory, may be of crucial diagnostic significance. In cases where the diagnosis remains in doubt in spite of competent blood study, a gastric analysis with histamine should be done and if free hydrochloric acid is found pernicious anemia can be excluded. In patients with achlorhydria the careful observation of the therapeutic response to refined liver extract according to the expectations outlined above is a practical method of proving the diagnosis.

SUMMARY

Four types of disturbed neurologic function occur in pernicious anemia (1) cerebral, (2) olfactory, (3) peripheral nerve and posterior columns of the spinal cord, and (4) lateral columns of the spinal cord. The neurologic prognosis depends upon the specific type of disability present, its severity, duration, and the adequacy of treatment. With intensive use of highly potent liver extracts intramuscularly the prognosis is far better than generally recognized. Serious neurologic residuals can be avoided if the diagnosis is made reasonably early.

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STANDARDIZATION OF THE QUICK PROTHROMBIN TEST

WITH REFERENCE TO THE STATISTICAL SIGNIFICANCE OF VARIATIONS IN THE PROTHROMBIN CONCENTRATION WITH USE OF A STABLE THROMBOPLASTIN OF HIGH POTENCY

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WITH increasing use of tests of the prothrombin concentration as an aid in the diagnosis of diseases of the liver and as a guide in vitamin K and dicumarol therapy,¹⁻⁶ it is imperative that the determinations be accurate. There are many ways of measuring the prothrombin concentration and each admits of numerous sources of error. Even when the most careful technic is practiced, appreciable errors in determining the prothrombin concentration may occur unless the thromboplastin used in the test is properly standardized. Furthermore, the results of the test may be misinterpreted unless the amount of variation due to chance alone is appreciated. The present study was undertaken in order to develop an accurate method of standardization of thromboplastin and to investigate the range of normal variation in the results of the Quick prothrombin test.

The Quick prothrombin test⁷ is an indirect measurement of the plasma prothrombin concentration. It is based on the theory of blood coagulation proposing that thromboplastin in the presence of calcium converts prothrombin to thrombin, which in turn converts fibrinogen to fibrin, the strands of which are the end results of clotting. With rare exceptions, the variations in the concentration of calcium and fibrinogen encountered in man do not affect the blood coagulation time. Therefore the speed of the reaction, after addition of an excess of thromboplastin, is used as a measure of the amount of prothrombin present. The standard of reference is the time required for the reaction to take place in the *normal* subject.

Quick⁷ states that the prothrombin concentration in the blood of healthy adults is remarkably constant and this normal level can therefore be designated as 100. He gives a table of prothrombin times corresponding to the various prothrombin concentrations. These values are apparently based on tests made on plasma dilutions in a single normal subject. When the blood of one subject is used as a standard of normality, it is implied that similar values would be obtained in tests made on the blood of any random normal subject. One of the purposes of the present investigation is to determine whether there are any significant differences in the prothrombin concentration in different normal subjects, and, if such differences do exist, to estimate the number of normal subjects required to establish reliable mean prothrombin values.

Quick further states that with an active preparation of thromboplastin the coagulation time is 11 to 12½ seconds. Yet in his conversion table the time of 12½ seconds corresponds to a plasma prothrombin concentration of 80 per cent. If one

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interprets the statement to mean that any specimen of thromboplastin that gives a prothrombin time of from 11 to 12.5 seconds in the plasma of a random normal subject, is a satisfactory reagent, it is obvious that it is still necessary to standardize each individual specimen of thromboplastin. Quick's table is an example of a standardization of a given specimen of thromboplastin and is not valid for all specimens. Thus the advantage of preparing one large specimen of dehydrated brain substance and storing it so that it can be used over a long period of time without change in potency is obvious.

When a potent thromboplastin solution is used, the prothrombin time is very short and the range of prothrombin times corresponding to variations in the prothrombin concentration between 100 and 50 per cent of the mean normal is very small. This has led to the suggestion that the test be performed on diluted plasma. It has been assumed that if the prothrombin time were prolonged by means of dilution of the plasma, greater accuracy in timing the reaction could be attained and smaller differences in the prothrombin concentration would be apparent. Quick has recommended that the test be performed on plasma diluted to 50 per cent of its original concentration, but only if the undiluted plasma prothrombin concentration is above 50 per cent. Link⁸ prefers to perform the test on plasma diluted to a concentration of 12.5 per cent. In the technic used by Allen, Julian, and Dragstedt,⁹ the test is performed on ten serial dilutions of plasma with concentrations ranging from 50 to 5 per cent of the original undiluted plasma. We propose in the present investigation to determine the comparative accuracy of prothrombin tests performed on diluted and undiluted plasma.

The significance of variation in the prothrombin concentration in a single individual is of great practical importance. Lord and Andrus¹⁰ have stated that in jaundiced patients with prothrombin levels below 80 per cent of normal, a rise of 10 per cent or more within twenty-four hours after intramuscular administration of 2 mg. of menadione indicates that extrahepatic jaundice is present. Changes in the prothrombin concentration are also used as a therapeutic guide in the administration of dicumarol.^{6,8} In the present investigation the degree of normal variation in repeated tests of the prothrombin concentration at various prothrombin levels in the same normal subject is studied.

METHODS

1. *Preparation and Storage of Dehydrated Brain*—We have found that human brain obtained at autopsy is a more potent source of thromboplastin than the average rabbit brain available in this locality. Material obtained from a single human brain was used in this investigation. The pia was completely stripped from about 400 Gm. of the brain, which was then macerated under acetone in a glass mortar. The acetone was replaced twice, the final product being a granular nonadhesive powder, which was dried on a water suction filter. This material was placed in a loosely stoppered jar within a calcium chloride desiccator, which was then evacuated for five minutes by means of an oil suction pump. The evacuated desiccator and its contents were stored in the refrigerator. Whenever material was required for testing, the desiccator was opened, a portion of the specimen was removed, and the desic-

cator was again evacuated and stored. We have kept dehydrated brain specimens for several years under these conditions with no loss of potency. If human brain tissue is not available, a quantity of rabbit brain tissue could be pooled and stored in a similar manner. This procedure allows more uniform results and saves much time and material by eliminating the necessity of preparing and individually testing small amounts of dehydrated rabbit brain stored in separate evacuated ampules.

2. *Preparation of Blood Specimens*—The dry oxalate mixture (6 mg of ammonium oxalate and 4 mg of potassium oxalate per 5 cc of blood) recommended by Winrobe¹¹ for the erythrocyte sedimentation test has proved satisfactory also as an anticoagulant for the blood used in the Quick prothrombin test. Specimen tubes containing this dry mixture are easier to store for clinical use than tubes containing the solution of sodium oxalate used by Quick⁷; furthermore, a part of the specimen so obtained may be used for numerous other hematologic tests. We have continued to use the original calcium chloride concentration (0.025 mol) recommended by Quick.

After addition of the blood to be tested, the tubes were repeatedly inverted to insure proper mixing. The blood specimens were centrifugalized at 2000 revolutions per minute for ten minutes, and the supernatant plasma was used for testing. All determinations were completed within four hours after the blood was drawn.

3. *Preparation of Thromboplastin*—An amount of 0.3 Gm. of dehydrated brain was removed from the storage bottle and mixed with 5 cc of physiologic saline solution. The mixture was incubated at from 48 C. to 50 C. for fifteen minutes, during which time it was stirred every three minutes with a glass rod. After incubation the particulate matter was allowed to settle to the bottom of the tube and the supernatant fluid was used for testing.

4. *Determination of Prothrombin Time*—The tube containing calcium chloride solution was kept in the water bath at 37 C. To determine the prothrombin time, 0.1 cc of plasma was pipetted into a clean 12 by 100 mm glass tube, 0.1 cc of thromboplastin solution was added and the tube was placed in the water bath for one-half minute. * With the right hand, using a 1 cc pipet, † 0.1 cc of calcium chloride solution was added to the mixture, and at the same instant the stopwatch was started with the left hand. The tube was then quickly picked up with the left hand, momentarily agitated, and replaced in the water bath. After replacing of the calcium chloride pipet, the prothrombin testing tube was picked up with the right hand and tilted or twirled continuously while being held with its contents clearly visible yet barely submerged in the water bath. The instant the first strands of fibrin appeared, the watch was stopped with the left hand, and the prothrombin time of the sample was recorded to the nearest half-second. Determinations were made in triplicate and the results were averaged. All tests were performed by one of the writers (P. M. A.).

5. *Statistical Methods*—Standard methods were used for determining the mean,

* Quick recommends one minute but we have found one-half minute to be satisfactory if the calcium chloride is kept at 37 C.

† Quick recommends the use of a short serologic pipet and forcible blowing of the calcium chloride solution into the thromboplastin plasma mixture.

the standard error of the mean, and the standard deviation.¹² The mean ± 2 standard deviations was accepted as representing the range to be expected about any given point.

The manner of conversion of prothrombin times to prothrombin concentrations used in this study is admittedly imperfect. The variations in the prothrombin time of any group of normal plasmas are due partly to technical variables and partly to actual differences in the amount of prothrombin in the plasmas tested. Technical variables would theoretically produce an equal amount of variation on either side of the mean time. However, because the relationship of prothrombin concentration to prothrombin time is parabolic, a given time interval will correspond to progressively greater amounts of prothrombin, the shorter the prothrombin time becomes. Because of this, an equal deviation in time about any given point will not correspond to an equal deviation in the prothrombin concentration. The true prothrombin concentrations of a group of normal plasmas should fall into the theoretic normal curve of frequency distribution and the corresponding prothrombin times should form a skewed distribution curve. When, as in the present study, the situation is reversed and the prothrombin times are treated statistically as if they fell into the theoretic normal distribution, the prothrombin concentrations thus derived will form a skewed distribution curve. The value found for the mean prothrombin concentration in this manner is therefore less than the true value, and the variation in the prothrombin concentrations about the mean is not equal, as it should be, but is greater above than below the mean.

Despite the inherent error of the method, we have treated the data in this study as if the prothrombin times fell into the theoretic normal distribution. This was done in order that the results of the Quick prothrombin test, as it is ordinarily performed in the average clinical laboratory, could be interpreted in relation to the findings here reported. We are fully aware of the fact that the prothrombin concentrations given in this report are not the same as would be found if it were possible to determine directly the amount of prothrombin in the plasma.

It is necessary to have standards of reference to which the results of every investigator can be referred. The use of the prothrombin time itself is unsatisfactory, because of differences in the potency of the thromboplastin specimens employed and because of variations in the technic of performing the test. For these reasons, the conversion of prothrombin time to prothrombin concentration was originally suggested by Quick. Perhaps it would have been better to have expressed the variations from normal in statistical terms, so that degrees of abnormality could be expressed in terms of the number of standard deviations from the mean prothrombin time.

MATERIAL STUDIED

Determinations of the prothrombin time were made on the undiluted plasma specimens (100 per cent) and on specimens diluted with physiologic saline solution to 75, 50, and 25 per cent of their original concentrations. One series of tests was made on the blood of each of 30 normal subjects, and a similar series of tests was repeated thirty times on the blood of a single normal control (P. M. A.) This

latter individual was chosen as the standard control because in preliminary experiments his prothrombin time was found to be very close to the mean prothrombin time of a group of normal subjects. A separate aliquot of the dehydrated brain specimen was used for each paired series of tests on one member of the normal group and on the standard control. Eight additional series of determinations were made on the bloods of each of two individuals within the normal group. These were chosen for special study because the prothrombin time of one of them (A A) was among the shortest and that of the other (M R) among the longest found in the group.

RESULTS

1 *Distribution of Normal Prothrombin Times in Diluted and Undiluted Plasma*—The distribution of prothrombin times of the various plasma dilutions is given in figure

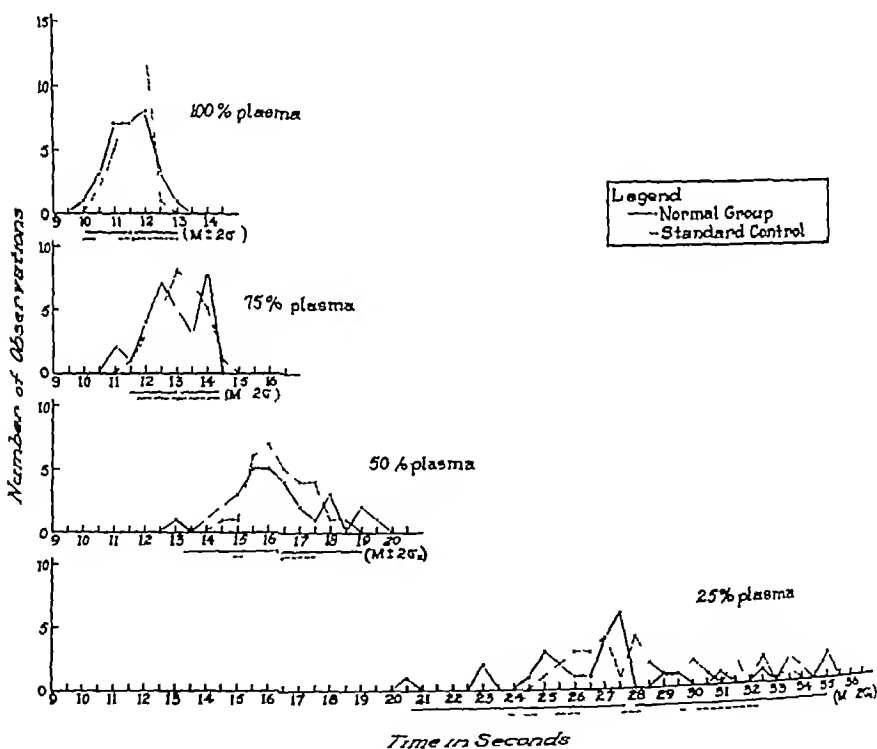


FIG. 1. DISTRIBUTION OF PROTHROMBIN TIMES IN VARIOUS PLASMA DILUTIONS IN NORMAL GROUP AND IN STANDARD CONTROL.

1 and statistical analyses of the data are given in table 1. The prothrombin times do not appear to form skewed distribution curves as would be expected, because the step intervals are too long and the number of observations are too few. In the normal group the mean prothrombin time was 11.5 seconds and the standard deviation 0.73 seconds. The normal range ($M \pm 2\sigma_x$) was from 10.04 to 12.96

seconds. The mean time for this specimen of dehydrated brain therefore falls within the range specified by Quick for an acceptable specimen. It should be noted, however, that our mean times for 50 and 25 per cent plasma are somewhat longer than those given by Quick.

When undiluted plasma was used the standard deviation of the prothrombin time was almost identical in the standard control and in the normal group. This would appear to indicate that the entire variation in the prothrombin time of the normal group was due to technical variables. However, in the tests made on diluted plasma, the standard deviation was less in the standard control than in the normal group, and although the difference is not great, it suggests that some of the differences in the prothrombin times of individuals in the normal group were due to differences in the prothrombin content of their plasmas.

TABLE 1—*Prothrombin Times (in Seconds) of the Normal Group and of the Standard Control Found in 100, 75, 50, and 25 Per Cent Plasma*

	100% Plasma		75% Plasma		50% Plasma		25% Plasma	
	Normal group	Standard control	Normal group	Standard control	Normal group	Standard control	Normal group	Standard control
Number of observations (N)	30	30	30	30	30	30	30	30
Mean (M)	11.50	11.59	12.95	13.10	16.26	16.43	27.84	28.32
Standard error of mean (σ_M)	0.13	0.14	0.14	0.12	0.28	0.16	0.65	0.41
Standard deviation (σ_x)	0.73	0.75	0.75	0.64	1.51	0.87	3.57	2.23
Lower limit of normal (M - $2\sigma_x$)	10.04	10.09	11.45	11.72	13.24	14.69	20.70	23.86
Upper limit of normal (M + $2\sigma_x$)	12.96	13.09	14.45	14.38	19.28	18.17	35.08	32.78

The standard deviation of the prothrombin time was markedly increased in the diluted plasma specimens both in the normal group and in the standard control. However, the significance of this finding is not obvious unless the prothrombin times are converted to prothrombin concentrations.

2. *Distribution of Normal Prothrombin Concentrations in Diluted and Undiluted Plasma*—With the assumption that the prothrombin concentration is equivalent to the plasma dilution, a graph was constructed in which the ordinate represented the prothrombin time and the abscissa the prothrombin concentration. A smoothed curve was plotted on the graph, using the mean prothrombin times of the plasma dilutions of the normal group. In order to complete the curve, additional prothrombin determinations were made on the 10 and 5 per cent plasma specimens from ten members of the normal group (fig. 2). By readings from this curve, prothrombin concentrations were assigned to the various prothrombin times (table 2). Analysis of these data shows that although the normal range of prothrombin times is much smaller in undiluted plasma, the range of prothrombin concentrations is very much greater, while conversely, although the range of prothrombin times is greater in diluted plasma, the range of prothrombin concentrations is much smaller.

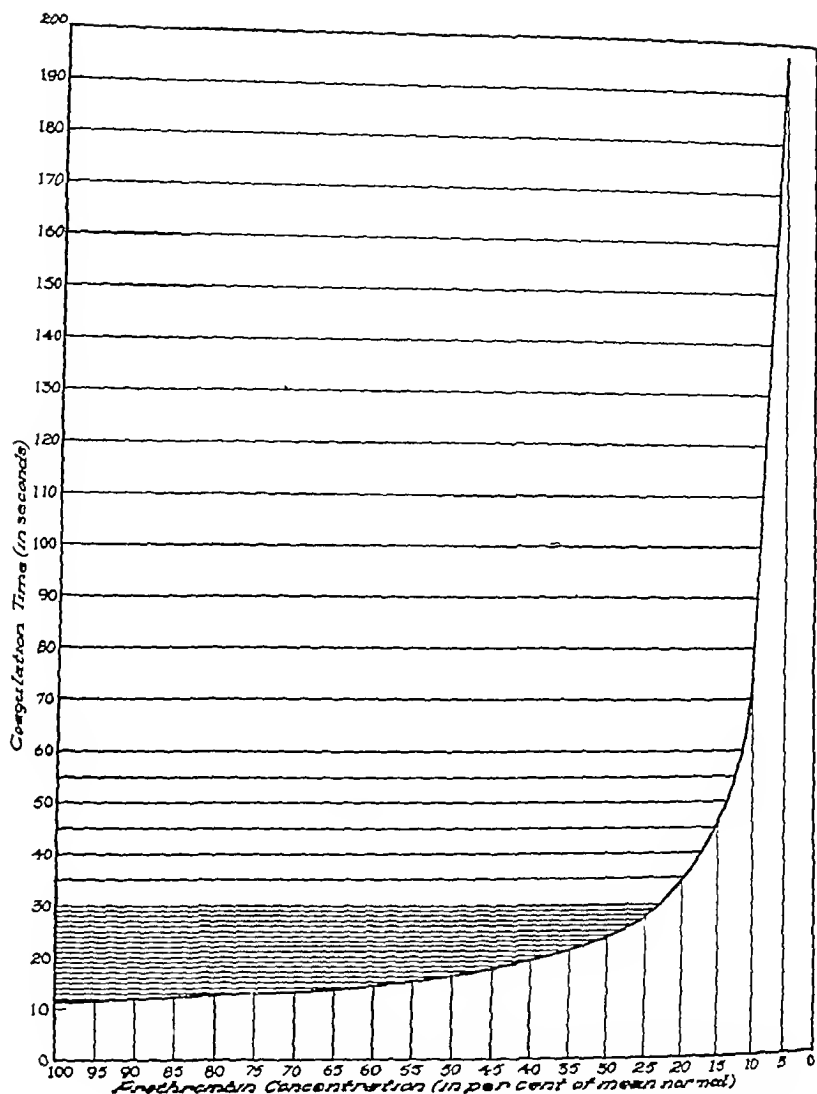


FIG. 2. CURVE USED FOR CONVERSION OF PROTHROMBIN TIME TO PROTHROMBIN CONCENTRATION

TABLE 2.—Prothrombin Concentrations (in Percentage of Mean Normal) of the Normal Group and of the Standard Control Found in 100, 75, 50, and 25 Per Cent Plasmas

	100% Plasma		75% Plasma		50% Plasma		25% Plasma	
	Normal group	Standard control	Normal group	Standard control	Normal group	Standard control	Normal group	Standard control
Number of observations (N)	30	30	30	30	30	30	30	30
Mean (M)	100	97.5	75	73.5	50	49	25	24.5
Lower limit of normal ($M - 2\sigma_x$)	75	73.0	58	58.0	39	42	19	21.0
Upper limit of normal ($M + 2\sigma_x$)	+100	+100.0	+100	96.0	70	56	35	29.0

3 *Comparison of Accuracy of Tests on Diluted and Undiluted Plasma*—An attempt was made to compare the accuracy of the results obtained in testing diluted and undiluted plasma by multiplying the prothrombin concentrations found in the diluted specimens by their dilution factors. The values for the mean and the normal range of the prothrombin concentrations obtained in this manner are given in table 3. In the normal group there was no significant difference in the values obtained for the lower limit of normal in any of the plasmas. The values for the upper limit of normal were identical (140 per cent) in the 25 per cent and 50 per cent plasma specimens, but in the 75 and 100 per cent plasmas the prothrombin concentrations were at an indefinite value over 100 per cent and therefore could not be compared with the values obtained in the more dilute plasmas. For all practical purposes, however, it appears that there was no difference between the results obtained in diluted and in undiluted plasma in the normal group.

TABLE 3.—*Comparison of the Prothrombin Concentrations (in Percentage of Mean Normal), in Diluted and Undiluted Plasma, of the Normal Group and of the Standard Control*

	Normal Group				Standard Control			
	Plasma							
	100 %	75 %	50 %	25 %	100 %	75 %	50 %	25 %
	Value	Value × 1.33	Value × 2	Value × 4	Value	Value × 1.35	Value × 2	Value × 4
Number of observations (N)	30	30	30	30	30	30	30	30
Mean (M)	100	100	100	100	98	98	98	98
Lower limit of normal ($M - 2\sigma_x$)	75	77	78	76	73	77	84	84
Upper limit of normal ($M + 2\sigma_x$)	+100	+133	140	140	+100	128	112	116

In the standard control there was no significant difference between values for the upper or lower limit of normal obtained in 25 and 50 per cent plasma. In the 75 and 100 per cent plasma the lower limit of normal was somewhat lower than in the more dilute samples. In the 75 per cent plasma the upper limit of normal was considerably higher than in the 50 and 25 per cent samples. In the undiluted plasma the upper limit of normal was at an indefinite value over 100 per cent and therefore could not be compared with the results obtained in the diluted plasma samples. From these results it appears that repeated tests on the same normal individual may be more accurate if done on plasma diluted to at least 50 per cent of its original concentration.

4 *Tests for Variation in Potency of Thromboplastin*—The differences in the prothrombin values in the standard control found at different times may be due to a number of factors, including variation in the preparation of thromboplastin, in obtaining the blood specimen, and in timing the reaction. In addition, there may even be slight fluctuations in the amount of prothrombin in the plasma of a normal subject at different times. In order to determine whether there was any variation in the potency of the thromboplastin solution here employed, we adopted a procedure suggested by Quick¹ for comparing the potency of an unstandardized thromboplastin solution with that of a standardized specimen. By this method the

prothrombin time is determined on the blood of a normal subject, using the unstandardized specimen of thromboplastin, the percentile prothrombin concentration that would have been assigned to this prothrombin time had the blood been tested with the standardized thromboplastin solution is determined, the same percentage value is assigned as the potency of the unstandardized thromboplastin solution, and all determinations of the prothrombin concentration on unknown specimens of plasma made with it are so corrected. In using this method one must make the assumption that any normal subject always has exactly 100 per cent prothrombin in his plasma and that there are no variables in determining the prothrombin time other than the potency of the thromboplastin solutions employed. As has been stated above, there appear to be actual differences in the prothrombin concentration of normal subjects, but this objection to the method can be obviated by using as the standard control a normal subject whose mean prothrombin time is known to be the same as, or reasonably close to, the mean prothrombin time of a large group of normal subjects.

TABLE 4—*Values for the Prothrombin Concentrations (in Percentage of Mean Normal) Obtained by the Half-Dilution Technique in the Normal Group*

	Uncorrected Values	Corrected Values
Number of observations (N)	30	30
Mean (M)	101.5	103.1
Standard error of mean (σ_M)	2.3	2.4
Standard deviation (σ_x)	12.9	13.3
Lower limit of normal ($M - 2\sigma_x$)	75.7	76.5
Upper limit of normal ($M + 2\sigma_x$)	127.3	129.7

The values originally found are compared with those obtained when individual prothrombin concentrations were corrected for possible variations in the potency of the thromboplastin solution.

The thirty aliquots of thromboplastin solution used in this study (each made from the same specimen of dehydrated brain) were assigned a potency equivalent to the prothrombin concentration of the standard control as determined in each specimen. The values for the prothrombin concentration of the normal group were then corrected according to the potency value of the thromboplastin with which each plasma was tested. All determinations of the prothrombin concentration were calculated from the 50 per cent plasma values. The corrected and uncorrected determinations are given in table 4. There were no significant differences between them. The prothrombin concentration was slightly over 100 per cent in the uncorrected group, owing to unavoidable errors in smoothing the standardization curve and in reading the prothrombin concentration from it. The mean value in the corrected group was higher than that in the uncorrected group because the mean prothrombin concentration of the standard control was only 97.5 per cent. It is therefore apparent that with the methods of storage of dehydrated brain and preparation of thromboplastin solution here employed, no correction for variability in the potency of thromboplastin is necessary or valid.

5 *Comparison of Prothrombin Values in Two Selected Normal Subjects*—On the basis of the data present in table 3, it would appear that the normal range of prothrombin concentrations is from 75 to 140 per cent. In order to show more clearly the difference in the prothrombin concentrations of different individuals, further studies were carried out on two members of the normal group. The first, A A, was chosen because his prothrombin time was the fastest and the second, M R, because her prothrombin time was the slowest found in the normal group. The prothrombin times in the various dilutions show a significant difference between the two plasmas (table 5). When the prothrombin times were converted to prothrombin concentrations, it was found that the mean value for M R in the undiluted speci-

TABLE 5—*Comparison of the Prothrombin Times (in Seconds) of 2 Selected Normal Subjects*

	100 % Plasma		75 % Plasma		50 % Plasma		25 % Plasma	
	A A	M R	A A	M R	A A	M R	A A	M R
Number of observations (N)	9	9	9	9	9	9	9	9
Mean (M)	10 80	12 50	11 80	14 10	14 60	18 30	24 50	33 50
Standard error of mean (σ_M)	0 12	0 13	0 22	0 16	0 20	0 32	0 57	0 46
Standard deviation (σ_x)	0 37	0 40	0 65	0 47	0 61	0 95	1 70	1 37
Lower limit of normal ($M - 2\sigma_x$)	10 10	11 70	10 60	13 20	13 40	16 40	21 10	30 80
Upper limit of normal ($M + 2\sigma_x$)	11 60	13 30	13 10	15 00	15 80	20 20	27 90	36 20

TABLE 6—*Comparison of Prothrombin Concentrations (in Percentage of Mean Normal, Using 50 Per Cent Plasma and Multiplying by Dilution Factor) of 2 Selected Normal Subjects*

	A A	M R
Number of observations (N)	9 0	9 0
Mean (M)	116 8	83 3
Standard error of mean (σ_M)	2 8	1 6
Standard deviation (σ_x)	8 5	4 8
Lower limit of normal ($M - 2\sigma_x$)	99 7	73 8
Upper limit of normal ($M + 2\sigma_x$)	133 8	92 9

men was 84 8 per cent and the standard deviation 7 6 per cent, but the mean value for A A was over 100 per cent and the two sets of observations could not be directly compared. The prothrombin concentrations obtained by doubling the values in the 50 per cent plasmas (table 6) show more clearly the extent of the difference between the two plasmas, the mean prothrombin concentration for A A being 116 8 per cent and for M R 83 3 per cent of the mean of the normal group.

6 *Comparison of Accuracy of Tests on Diluted and Undiluted Plasma in Normal Subject*—A comparison of the prothrombin concentrations obtained for M R in undiluted plasma and by doubling the values for her 50 per cent plasma (table 7) shows that there is no significant difference in the mean values of the prothrombin concentration obtained by the two methods. The standard error of the mean and

the standard deviation of the prothrombin concentration were greater in the tests made on undiluted plasma than in those made on diluted plasma, and although the difference is not statistically significant, it again suggests that repeated tests on the same individual may be more accurate if diluted plasma is employed.

7 *Size of Sample Required for Accurate Standardization of Thromboplastin*—It is apparent from the data presented that the use of a single series of determinations on the blood of one random normal subject as a normal standard may lead to considerable error. An unknown prothrombin that would be reported as 100 per cent if the lowest values found for M. R. were used as a standard, would be reported as 55 per cent if the highest values found for A. A. were used as a standard. The implications of such an error in interpreting the response to administration of vitamin K or in regulating dicumarol therapy are obvious. However, the range of normal prothrombin times is so small that it probably would not require a group of as many as 30 subjects, such as was used in this study, to establish reliable values.

TABLE 7—*Prothrombin Concentrations (in Percentage of Mean Normal) of M. R. Obtained with Undiluted Plasma Compared with Those Found by the Half Dilution Test*

	100% Plasma	50% Plasma
	Value	Value X 2
Number of observations (N)	9	9
Mean (M)	84.8	83.3
Standard error of mean (σ_M)	2.5	1.6
Standard deviation (σ_x)	7.6	4.8
Lower limit of normal ($M - 2\sigma_x$)	69.6	73.8
Upper limit of normal ($M + 2\sigma_x$)	100.0	91.9

In order to determine how small a sample would produce reliable data, we used the following procedure. The standard error of the mean (σ_M) of the normal group ($N = 30$) was arbitrarily set at three times its actual value. The mean (M) was still well above three times its own sampling error. With this figure as the standard error of the mean, the formula

$$\sigma_M = \frac{\sigma_x}{\sqrt{N-1}}$$

was used ($N - 1$ was employed as a precaution because of the small size of the sample). This technic applied to the figures obtained gave the following results: with 100 per cent plasma, $N = 4.5$, with 50 per cent plasma, $N = 4.2$, with 25 per cent plasma, $N = 4.4$. It therefore appears that a sample of 5 normal subjects would be sufficient to establish a reliable mean. However, these results must be interpreted with caution, because this statistical technic carries with it the assumption that the conditions of the experiment would be the same as those established for the tests made on the group of 30 normal subjects originally studied.

DISCUSSION

There has been much discussion of the relative merits of various methods of determining the prothrombin concentration when the tests are performed by ex-

perienced observers under ideal conditions, but scant attention has been given to the matter of the reliability of results reported by the average clinical laboratory. Errors in performing the test may obscure the diagnosis of liver disease or may lead to serious errors in the administration of dicumarol.

The principal sources of error in all methods of testing the prothrombin concentration are lack of experience with the test and inadequate standardization of the thromboplastin used. Precise technic can be achieved only by practice and strict attention to the details of the test. The results obtained in this study clearly indicate that even with the most precise technic, great errors may be made in the Quick test if a single specimen of blood from one random normal subject is used as a normal standard. Our results suggest that the blood of at least 5 normal subjects should be used if reliable mean values are to be established. With the present sources of thromboplastin available to the average clinical laboratory, such care in standardization is not possible. One solution of the problem would be for each laboratory to accumulate gradually a pool of dehydrated rabbit brains and to preserve the specimen in an evacuated desiccator as was done in this study. If 20 brains were so pooled, proper standardization with 5 normal blood specimens would require only one-fourth of the effort that would be spent in the inadequate standardization of each brain with a single blood specimen. Another solution would be for commercial distributors to market material from a single pooled brain specimen in sets containing sufficient dehydrated brain in evacuated ampules to allow for proper standardization as well as for a sizable group of individual prothrombin determinations.

Our results suggest that slightly greater accuracy may be attained if the test is performed on plasma diluted to 50 or 25 per cent of its original concentration. Our data unfortunately do not permit a comparison of the accuracy of tests made on the 12.5 per cent plasma concentration suggested by Link.⁸ A distinction should be made between the inherent error of the test when it is performed with the greatest possible accuracy, and gross errors in timing the reaction that are due to poor technic. It is apparent from an examination of the prothrombin concentration curve (fig. 2) that when undiluted plasma is used, an error of one second in timing the reaction may result in an error of 20 per cent or more in the value obtained for the prothrombin concentration. If the same plasma were diluted to 25 per cent of its original concentration, a similar error of one second in timing would result in an error of approximately 1 per cent in the value obtained for the prothrombin concentration. When this error is multiplied by 4 in the final calculation of the prothrombin concentration, it is still significantly less than the error of the test performed on undiluted plasma. However, minimizing of errors that can be eliminated by precise methods does not constitute a valid reason for attributing superiority to the dilution technic.

From the results of tests made on diluted plasma, in the standard control, it is apparent that an elevation of 10 per cent in prothrombin in a single individual after the administration of vitamin K becomes significant only if the prothrombin concentration is 25 per cent or less. When the prothrombin concentration is in the neighborhood of 50 per cent, a change of 15 per cent would be required for statistical demonstration that a significant change in concentration had oc-

curred, and when the prothrombin concentration is in the neighborhood of 75 per cent, even an elevation to a concentration of 100 per cent cannot be proved to be statistically significant. This variation due to chance alone could be considerably decreased if the means in two groups of determinations made respectively before and after administration of vitamin K were compared. The same limits of significance would apply in reverse order to changes in the prothrombin concentration following administration of dicumarol.

SUMMARY

A large specimen of human brain, obtained at autopsy, was dehydrated and stored in an evacuated desiccator. Portions of the material were removed when needed and the desiccator was re-evacuated. Under these conditions there was no loss of thromboplastic potency over a period of two years.

TABLE 8

	100 % Plasma	50 % Plasma	25 % Plasma
	Value	Value X 2	Value X 4
Normal prothrombin time			
mean	11.5 sec.		
lower limit	10.0 sec.		
upper limit	13.0 sec.		
Normal prothrombin concentration			
mean	100%	100%	100%
lower limit	75%	78%	76%
upper limit	+100%	140%	140%

TABLE 9

Mean value (%)	Minimum (%)	Maximum (%)	Total variation (%)
97.5	73	+100	+27
73.5	58	96	38
49.0	42	56	14
24.5	21	29	8

The dry oxalate mixture (6 mg. of ammonium oxalate and 4 mg. of potassium oxalate) was used as an anticoagulant in order to obtain plasma for the prothrombin tests.

With the specimen of thromboplastin used in these experiments, normal standards for the Quick prothrombin test were found as shown in table 8. These results do not necessarily mean that the normal range of prothrombin concentrations is from 75 to 140 per cent of the actual amount of prothrombin in the plasma of the average normal subject. They mean only that with the methods here employed, values within this range may be expected to occur.

There was no significant difference between the prothrombin values obtained

in a group of normal subjects by methods using diluted or undiluted plasma. In repeated tests on the same subject there appeared to be slightly greater accuracy when the tests were performed on diluted plasma, but there was no significant difference between the results obtained with plasma diluted to 50 or to 25 per cent of its original concentration.

Repeated tests on diluted and undiluted plasma from the same normal subject produced the range of normal variation about the various prothrombin concentrations shown in table 9.

The normal variation that may be expected to occur at any given prothrombin level must be considered in interpreting the variations that appear after administration of vitamin K or dicumarol.

The use of a single specimen of blood from one random normal subject as a normal standard may lead to considerable error, because of unavoidable technical variables and because of differences in the prothrombin concentrations of different normal subjects. To establish reliable normal standards, the blood of at least 5 normal subjects should be individually tested with the same specimen of thromboplastin and the results averaged.

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INDIVIDUAL BLOOD DIFFERENCES IN RELATION TO PREGNANCY, WITH SPECIAL REFERENCE TO THE PATHOGENESIS OF PREECLAMPTIC TOXEMIA

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PREECLAMPTIC toxemia is recognized as an important cause of maternal death and is often a source of permanent ill health in a woman surviving an attack. The disease presents a striking clinical picture, consisting of sudden appearance of proteinuria, hypertension and edema during the last trimester of pregnancy and equally rapid improvement of the patient after parturition. This spectacular course and the sometimes serious results have tempted many workers to study the pathogenesis of this condition.

Among the many explanations offered for preeclamptic toxemia is the theory that isoimmunization of the mother to agglutinogens in the red cells of the fetus is the cause of eclampsia. Originally suggested by Dienst¹ in 1905, this theory was abandoned by the same investigator three years later.² The idea was revived in 1923 by McQuarrie³ and Ottenberg,⁴ only to be discredited again by other workers. Today it is not even mentioned in most of the standard textbooks on obstetrics.

The isoimmunization theory of the pathogenesis of eclampsia was first suggested by the similarity of the symptoms and pathologic changes of this disease to those of experimental hemolytic transfusion reactions produced in animals by injections of blood of other species. According to the theory as it was first advanced by Dienst, two conditions were necessary for eclampsia to occur: 1. There had to be a defect at the placental site permitting fetal blood to gain access to the maternal circulation; 2. The red cells of the fetus had to be incompatible with the serum of the mother. Dienst attempted to demonstrate both of these points. To detect the presence of a communication between the circulations of the fetus and the mother he injected methylene blue (methylthionine chloride) into the umbilical vessels and examined the maternal urine for presence of the dye. Among 160 women he found 32, or 20 per cent, whose urine turned blue after this injection. For testing serologic incompatibility he matched the blood of 118 mothers and their respective children in 1,726 combinations. He found that the serum of 24 of these mothers agglutinated and eventually lysed the red cells of their fetuses. In 15 of these 24 women he found that the placenta did not permit the passage of methylene blue and there was no trace of albumin in the urine. Of the remaining 9 women with blue urine, 7 had eclampsia and 2 had albuminuria. According to the view of Dienst, if the maternal serum contains antibodies (agglutinins or hemolysins) of

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high titer for the fetal red cells and if small amounts of fetal blood enter the mother's circulation through a defect in the placenta, the fetal cells will be clumped, blocking the arterioles of the mother.

The early investigations on this problem were hampered by the then limited knowledge of the individual differences in human blood. In Dienst's article no reference is made even to the four standard blood groups, his conclusions being based entirely on cross-matching tests. In McQuarrie's paper the data presented concerning the blood-grouping tests are inadequate and difficult to interpret according to present standards. Since these papers were written, knowledge concerning the four blood groups has advanced considerably, and, in addition, further individual differences in human blood have been discovered, of which the most important clinically are the Rh and Hr factors.⁵ The reinvestigation of this problem in the light of the more modern knowledge of blood grouping seemed, therefore, to offer a promising field for research.

The present study was set up to test several possibilities: 1. Evidence was sought as to whether pregnancy itself could cause a rise in isoagglutinin titer. 2. The possibility was considered that the process of parturition, by making it possible for fetal blood or other products to pass the placenta, might cause a rise in maternal isoagglutinin titer. To explore this possibility blood taken from each patient eight days post partum was examined and compared with that originally studied. 3. Finally patients with toxemia were studied in a similar manner to determine whether there was a greater incidence of incompatibility between mother and fetus, a higher titer of isoagglutinins at labor or a greater rise following delivery.

MATERIALS AND METHODS

A total of 183 normal mothers and their infants were studied, together with 35 patients with toxemia and their offspring. The normal women and most of those with toxemia were patients from the obstetric service of Bellevue Hospital. Material from several patients with toxemia was obtained through other hospitals.*

The scope of the study included an investigation of the standard blood groups, subgroups, M-N types, Rh types and Hr reactions in the entire series of mothers and newborn infants. At the time of delivery a sample of blood was obtained by venipuncture from the mother and a sample of the infant's blood was obtained from the vessels of the umbilical cord. A second sample of blood was usually taken from the mother on the eighth day post partum, the day before the one on which the patient was generally discharged from the hospital. In cases in which the tests on the umbilical cord blood gave unsatisfactory reactions, a fresh sample of blood was obtained from the infant by puncturing the heel or great toe and collecting 10 to 20 drops of blood in a small test tube containing 3 drops of 3.8 per cent sodium citrate solution. Additional tests were carried out on a number of the mothers at various times before and after delivery, but for simplicity the results of these tests are omitted from the tables.

The bloods from every mother and child were classified for the blood groups, subgroups and M-N types, and whenever possible the Rh and Hr types were also determined. At times when the supply of special Rh and Hr serums was low the bloods were merely tested with standard Rh serum and classified as Rh positive or Rh negative. The technique of these determinations is described elsewhere⁶⁻⁸ and will not be repeated here. With regard to the subgroups of group A, it should be mentioned that this test is not entirely reliable in the case of newborn infants.⁶ Because of the low sensitivity of infant blood to agglutination by absorbed B serum only positive reactions are reliable. Accordingly, in the tables,

*Dr. C. E. Heaton of French Hospital and Dr. M. E. Goldblatt of French Hospital and Manhattan General Hospital cooperated with us in this.

when the absorbed B serum agglutinated the infant's blood the subgroup was diagnosed as A₁ but when no clumping occurred no diagnosis as to subgroup was recorded

The serum of each mother was titrated to determine the content of anti A and/or anti B isoagglutinins. These titrations were done on blood taken at the time of delivery and also on the sample obtained one week post partum, in order to determine whether there had been any change in agglutinin titer. In some of the cases in which the woman's blood was Rh negative, her serum was also tested for presence of Rh antibodies

A few remarks concerning the technic of titrating for isoagglutinins appear to be necessary. Despite an experience with thousands of such titrations, the maximum titer we have ever encountered even in an immunized person, amounted to only several thousand units. We have never obtained any titer approaching the extravagant values reaching as high as several millions, claimed by certain workers. The only way we can account for the results reported by these workers is that the technic of rinsing the pipet used for preparing the serum dilutions might have been inadequate, so that serum was carried over from one dilution to another, or that if the same pipet was used for the test blood suspensions the cells might have become partly coated with antibody and thus been more susceptible to agglutination. To avoid these common pitfalls we have used the following precautions: 1. The pipet is rinsed three times with *fresh* saline solution between dilutions. The saline solution used for rinsing is distributed among a series of test tubes and a fresh tube of solution is used for each dilution (instead of the pipet's being rinsed in a common reservoir of saline solution which would soon become contaminated with serum). An alternative technic would be to change pipets between dilutions, but this is not necessary provided that the following additional precaution is observed: 2. Every serum giving a titer higher than 100 is retested as follows: A 1:10 or a 1:50 dilution is prepared, depending on the titer of the serum, by accurately measuring the proper volume of serum, with a graduated pipet, into an accurately measured volume of saline solution. Then with a *fresh* pipet the diluted serum is titrated. The titer of the concentrated serum is calculated simply by multiplying the titer obtained by the dilution factor. For example, if a claim of a titer of 5,000,000 units were correct, it should be possible to dilute the serum 1,000 times and find that this diluted serum still has a titer of 5,000. * No such control test was carried out by the investigators reporting the aforementioned extravagant titers.

In any case, it is obvious that the titer assigned to a serum is purely arbitrary and will vary with the method of titration used. If the identical technic is used by different workers, there is no reason why comparable results cannot be obtained. Our tests were carried out by mixing 1 drop each of the series of dilutions of the serum being titrated in a corresponding series of wells on a Boerner well slide with a drop of a fresh 2 per cent suspension of the standard test cells and a drop of saline solution. The slide was placed on a Boerner shaking machine for ten minutes and the reactions were then read with the naked eye and under the low power of a microscope. This method gives titers about one sixth as high as the values obtained in similar titrations set up in test tubes and read after standing for two hours at room temperature or after centrifugation for one minute at low speed.⁹

RESULTS

Mothers Without Toxemia—In table 1 are listed the findings in a series of 183 normal mothers and their newborn infants. Parallel columns show the blood groups of mothers and infants and the isoagglutinins in the mothers' serum at labor and at the end of the first week of the puerperium.

It may be of interest first to compare the isoagglutinin titers of these patients at term with the isoagglutinin titers of a random series of male professional donors. Accordingly, in table 2 we have summarized the agglutinin titers at the time of labor of the patients listed in table 1 and for comparison have listed the results of a series of titrations carried out by the identical technic and reported by Wiener and Moloney.¹⁰ The similarity between the distributions of the titer values in

* If serums with titers as high as 5,000,000 units actually existed 10 cc. of such a serum should be sufficient to group the bloods of the combined armed forces of all the United Nations!!

TABLE I—Cases of Mothers without Toxemia

Case No	Mother	Child	Titer of Maternal Serum for			
			A ₂ Cells		B Cells	
			At Labor	1 Wk P P	At Labor	1 Wk P P
1*	OMNRh ₀	ONRh-♀	8	48	12	24
2	ONRh ₁	OMNRh ₁ ♂	8†	8	24†	24
3	OMNRh ₁ Rh	OMNRh ₁ ♀	32†	48	12†	32
4	OMNRh ₂	ONRh ₁ Rh ₂ ♂	2	4	6	6
5	OMNRh ₁	OMNRh ₁ ♀	16	12	24	4
6‡	OMNRh ₁	OMRh ₂ ♂	16	16	32	64
7*‡	OMRh-	OMNRh ₂ ♂	2	2	8	8
8‡	OMRh-	OMNRh ₁ ♂	1	2	2	4
9‡	OMNRh ₁	ONRh ₁ ♀	4	12	24	24
10‡	OMRh-	OMNRh ₁ ♀	12	6	32	12
11	OMNRh ₁	OMNRh-♂	24	24	24	32
12*	ONRh ₁ Rh ₂	OMNRh ₁ Rh ₂ ♂ [‡] OMNRh ₁ ♂ [‡]	16	24	24	16
13	OMRh-	OMNRh ₂ ♀	4	4	4	4
14*	OMRh ₂	OMNRh ₂ ♂	24	24	24	64
15	OMNRh ₁	OMNRh ₁ ♀	16	10	8	10
16	ONRh ₁ Rh ₂	OMNRh ₂ ♂	2	2	4	8
17	OMNRh	OMNRh ₂ ♂	4	2	12	24
18 ‡	ONRh ₂	ONRh ₂ ♀	4	4	12	6
19	OMRh	OMNRh♂	2	4	2	4
20	OMRh ₁	OMRh ₁ ♀	4	4	4	8
21‡	ONRh ₁ Rh ₂	ONRh ₁ Rh ₂ ♀	16	48	8	32
22	OMNRh ₁	OMNRh ₁ ♂	4	2	6	8
23*	OMRh ₂	OMNRh ₁ Rh ₂ ♀	4	4	8	12
24*‡	OMNRh ₁ Rh ₂	OMNRh ₁ ♂	24	24	12	4
25‡	OMRh ₂	OMRh-♂	32	12	32	32
26	OMRh+	OMRh+♂	4	6	8	8
27‡	OMRh+	OMNRh+♂	4	4	4	4
28	OMRh+	OMRh+♂	2	2	24	6
29 ‡	OMNRh+	ONRh ₁ Rh ₂ ♀	4	8	4	4
30	OMRh ₂	OMRh ₁ Rh ₂ ♀	12	16	12	24
31	OMRh ⁺ Hr ⁺	OMRh-Hr+♂	12	16	4	8
32	ONRh ₁ Hr ⁺	ONRh ₁ Hr+♂	4	4	4	4
33‡	OMRh ₁ Rh ₂ Hr ⁺	OMNRh ₁ Hr-♂	6	6	4	2
34*	OMRh-Hr ⁺	OMNRh ₁ Hr+♂	6	6	6	8
35‡	OMNRh ₁ Rh ₂ Hr ⁺	OMNRh ₁ Rh ₂ Hr+♂	6	4	6	6
36‡	OMNRh ₂ Hr ⁺	OMNRh ₁ Hr+♀	4	12	4	12
37	OMNRh ₁ Hr ⁺	ONRh ₁ Hr-♂	6	6	6	8
38‡	OMRh ₁ Hr ⁺	OMNRh ₁ Hr+♀	4	6	16	12
39	OMNRh ₁ Hr-	OMNRh ₁ Hr-♀	6	4	4	6
40*	OMRh-Hr ⁺	OMRh ₁ Hr-♂	48	24	24	16
41	OMNRh ₁ Hr ⁺	OMRh ₁ Hr-♂	16	16	6	6
42‡	OMRh ₂ Hr ⁺	OMNRh ₁ Hr-♂	4	4	4	6
43	OMNRh ₁ Hr ⁺	OMRh ₂ Hr+♀	8	24	24	24
44‡	ONRh ₁ Hr-	ONRh ₁ Hr-♂	4	4	4	6
45	OMNRh ₁ Rh ₂ Hr ⁺	OMNRh ₁ Rh ₂ Hr+♀	8	12	4	6
46‡	OMNRh ₁ Hr-	ONRh ₁ Hr-♀	12	6	12	24
47‡	OMNRh-Hr ⁺	ONRh-Hr+♀	6	8	8	24
48‡	OMRh Hr ⁺	OMRh ₁ Hr-♀	6	16	6	16
49	OMRh ₂ Hr ⁺	OMRh ₂ Hr+♀	24	12	16	6
50	OMNRh ₁ Hr ⁺	OMNRh ₁ Hr+♂	6	4	4	6
51‡	OMNRh ₁ Hr ⁺	OMRh ₂ Hr+♀	4	4	6	6
52‡	OMRh ₁ Hr ⁺	OMRh ₁ Hr+♀	4	4	6	12
53	ONRh ₁ Rh ₂ Hr ⁺	OMNRh ₁ Hr-♂	6	6	12	6
54 ‡	OMNRh ₁ Rh Hr ⁺	ONRh ₁ Hr+♂	4	4	24	12
55‡	OMNRh ₁ Rh Hr ⁺	OMNRh ₁ ♀	2	6	6	6
56	ONRh ₁ Hr-	ONRh ₁ Hr+♀	6	12	6	12
57	OMNRh ₁ Rh ₂ Hr ⁺	OMNRh ₁ Hr-♀	12	6	4	4

TABLE I—Continued

Case No	Mother	Child	Titer of Maternal Serum to			
			A ₂ Cells		B Cells	
			At Labor	1 Wk P P	At Labor	1 Wk P P
58*	OMRh ₂ Hr ⁺	OMNRh ₂ Hr ⁺ ♀	2	4	4	12
59	OMRh ₂ Hr ⁺	OMNRh ₂ Hr ⁺ ♂		4	24	11
60	OMRh ₂ Hr ⁺	OMRh ₂ Hr ⁺ ♂	2	4	12	4
61§	OM\NRh ⁻ Hr ⁺	OMNRh ₂ Hr ⁺ ♀	8	12	48	64
62	A ₁ ΔNRh ₂	A ₁ ΔRh ₂ ♀			12	12
63	A ₁ ΔNRh ⁻	A ₁ ΔNRh ⁻ ♀			1	1
64§	A ₁ ΔNRh ₁	A ₁ ΔRh ₁ ♂			12	15
65	A ₁ ΔNRh ₁	A ₁ ΔNRh ⁻ ♀			32	15
66	A ₁ ΔNRh ₁	A ₁ ΔNRh ₁ ♂			8	6
67§	A ₁ NRh ₁	A ₁ NRh ₁ ♂			1	2
68	A ₁ ΔNRh ₂	A ₁ ΔNRh ₂ ♂			12	15
69*	A ₂ MRh ₁	A ₂ MRh ₁ ♀			12	8
70§	A ₁ ΔNRh ₁	A ₁ NRh ₁ ♂			16	4
71	A ₁ ΔNRh	A ₁ NRh ₁ Rh ⁻ ♂			16	10
72*	A ₁ ΔNRh ₁	A ₁ ΔNRh ₁ ♀			6	6
73	A ₁ NRh ⁻	A ₁ ΔNRh ⁻ ♀			4	4
74§	A ₁ ΔNRh ⁺	A ₁ NRh ⁺ ♀			8	4
75	A ₁ ΔNRh ⁺	A ₂ ΔNRh ⁺ ♂			4	2
76§	A ₁ NRh ⁺	A ₁ ΔNRh ⁻ ♀			12	8
77	A ₁ ΔNRh	A ₂ MRh ₂ ♀			32	32
78§	A ₁ ΔNRh ₁ Hr ⁺	A ₁ ΔNRh ₁ Hr ⁺ ♀			8	4
79§	A ₁ VRh ₁ Rh ₂ Hr ⁺	A ₁ MRh ₂ Hr ⁺ ♀			6	4
80	A ₁ NRh ₁ Hr ⁻	A ₁ NRh ₁ Hr ⁻ ♀			4	4
81*	A ₁ VRh ₂	A ₁ MRh ₂ Hr ⁺ ♀			6	12
82	A ₁ VRh ₂ Hr ⁺	A ₁ ΔNRh ⁻ Hr ⁺ ♂			12	12
83	A ₂ NRh ₁ Rh ₂ Hr ⁺	A ₂ ΔNRh ₁ Hr ⁺ ♀			16	12
84	A ₁ ΔRh ₁ Rh ₂ Hr ⁺	A ₁ VRh ₂ Hr ⁺ ♂			24	12
85	A ₁ VRh ₁ Hr ⁺	A ₁ VRh ₁ Hr ⁺ ♂			12	48
86	A ₁ ΔRh ⁻ Hr ⁺	A ₁ ΔRh ₁ Hr ⁺ ♀			6	6
87	A ₁ ΔVRh ₂ Hr ⁺	A ₁ ΔNRh ₁ Hr ⁺ ♀			24	24
88	A ₂ NRh ₁ Hr ⁺	A ₂ NRh ₁ Hr ⁺ ♀			12	6
89 *	A ₁ ΔNRh ₁ Hr ⁻	A ₁ ΔNRh ₁ Hr ⁺ ♂			4	4
90	A ₁ ΔMRh ₁ Rh ₂ Hr ⁺	A ₁ ΔMRh ₂ Hr ⁺ ♂			48	8
91	A ₁ ΔNRh ₂ Hr ⁺	A ₁ ΔNRh ₁ Rh ₂ Hr ⁺ ♀			6	8
92*§	A ₁ ΔNRh ₂ Hr ⁺	A ₂ MRh ₂ Hr ⁺ ♂			48	11
93*	A ₁ ΔNRh ₂ Hr ⁺	A ₁ VRh ₂ Hr ⁺ ♀			4	6
94	A ₁ ΔNRh ₂	ONRh ₂ ♀			6	8
95	A ₁ VRh ₁ Rh ₂	ONRh ₁ Rh ₂ ♂			32	32
96§	A ₁ ΔRh ₁	OMNRh ₁ ♂			8	12
97	A ₁ ΔNRh ⁺	OMNRh ⁺ ♀			16	12
98	A ₁ ΔNRh ⁻ Hr ⁺	OMNRh ⁻ Hr ⁺ ♀			64	48
99	A ₁ ΔNRh ₁ Hr ⁺	ONRh ⁻ Hr ⁺ ♀			96	9
100	A ₁ ΔRh ₁ Hr ⁺	OMNRh ⁻ Hr ⁺ ♀			6	4
101	A ₁ ΔNRh ₂ Hr ⁻	OMNRh ₁ Hr ⁺ ♂			12	12
102§	A ₁ ΔNRh ₁ Rh ₂ Hr ⁺	OMNRh ₁ Rh ₂ Hr ⁺ ♀			12	12
103	A ₁ ΔNRh ₁ Hr ⁺	OMRh ⁻ Hr ⁺ ♂			8	8
104	BΔNRh ₂	BVRh ₂ ♀	12	32		
105	B\NRh ₁	BNRh ₁ ♀	6	8		
106	BΔNRh ₁	BNRh ₁ ♂	4	2		
107	BVRh ⁻	BMRh ₁ ♂	4	6		
108§	B\NRh ₂	BΔNRh ₂ ♀	4	6		
109§	BVRh ₂ Hr ⁺	BΔNRh ₂ Hr ⁺ ♂	2	4		
110	BΔNRh ₁ Hr ⁺	BVRh ₁ Hr ⁺ ♀	6	12		
111	BΔNRh ₂ Hr ⁺	B\NRh ₂ Hr ⁺ ♀	12	12		
112	BΔNRh ₂ Hr ⁻	BΔNRh ₂ Hr ⁺ ♀	16	24		
113*§	BVRh ₂ Hr ⁻	BVRh ₂ Hr ⁺ ♀	24	12		
114*§	BVRh ₂	BVRh ₂ ♀	16	24		
115	BVRh ₂ Hr ⁺	BΔNRh ₂ Hr ⁺ ♀	6	4		

TABLE 1—Continued

Case No	Mother	Child	Titer of Maternal Serum for			
			A Cells		B Cells	
			At Labor	1 WL P P	At Labor	1 WL P P
116*	BNRh ₂ Hr ⁺	B\Rh ₁ Hr ⁺ ♀	6	12		
117*	BM\Rh ₂ Hr ⁺	B\Rh ₁ Hr ⁺ ♀	4	6		
118§	BMRh ₁ Hr ⁺	BMRh ₁ Hr ⁺ ♀	8	6		
119*	BMRh ₂ Hr ⁺	BMRh ₂ Hr ⁺ ♂	4	6		
120	BM\Rh ₂ Hr ⁺	BMRh ₁ Rh ₂ Hr ⁺ ♂	4	4		
121	BMRh ₁	OMRh ₁ ♂	2	2		
122	BM\NRh ₁	OM\NRh ₁ ♂	8	12		
123	BMRh ₂	OMRh ₂ ♀	24	12		
124	BM\NRh ₁ Rh ₂	OM\NRh ₁ ♀	1	1		
125	BM\NRh ₂ Hr ⁺	OM\NRh ₂ Hr ⁺ ♂	4	4		
126	BM\NRh ₂ Hr ⁺	OM\NRh ₁ Hr ⁺ ♂	12	24		
127*	BM\NRh ₁ Hr ⁺	OM\NRh ⁺ Hr ⁺ ♀	6	8		
128*	BMRh ₂	OMRh ₂ ♀	24	12		
129	A ₁ BMRh ₁	A ₁ BM\Rh ₂ ♀				
130§	A ₁ BMRh ₂	A ₁ BM\NRh ⁺ ♂				
131	A ₁ BMRh ⁺	A ₁ MRh ⁺ ♂				
132§	A ₁ B\Rh ₁ Hr ⁺	A ₁ BM\Rh ₁ Hr ⁺ ♀				
133	A ₁ BM\NRh ⁺ Hr ⁺	A ₁ \Rh ⁺ Hr ⁺ ♀				
134	A ₁ BM\NRh ₁ Rh ₂ Hr ⁺	A ₁ \NRh ₁ Rh ₂ Hr ⁺ ♂				
135	A ₁ BMRh ⁺	AMRh ⁺ ♀				
136	A ₁ BNRh ₁ Hr ⁺	AN\Rh ₁ Rh ₂ Hr ⁺ ♂				
137	A ₁ BMRh ₁ Rh ₂	AMRh ⁺ ♀				
138§	A ₁ BM\NRh ₁	BM\NRh ₁ Rh ₂ ♀				
139	A ₁ B\NRh ⁺	B\NRh ₁ ♀				
140§	A ₁ BM\NRh ⁺	BM\NRh ⁺ ♀				
141	A ₁ BMRh ₁ Hr ⁺	ABM\NRh ₁ Hr ⁺ ♀				
142 §	A ₂ BMRh ₂	AM\NRh ₁ ♀				
143	A ₂ BM\NRh ⁺	AN\Rh ⁺ ♂				
144§	A ₂ BM\NRh ₁ Hr ⁺	AB\NRh ₁ Hr ⁺ ♂				
145	A ₂ BN\Rh ₁ Hr ⁺	A ₂ BM\NRh ₂ Hr ⁺ ♀				
146§	ONRh ₁ Rh ₂	A ₁ BM\NRh ₁ Rh ₂ ♂	1	4	8	12
147	OM\NRh ₁	A ₁ BM\NRh ₂ ♀	6	12	8	12
148	OM\NRh ₁	ANRh ⁺ ♂	4	6	24	24
149§	OMRh ₁	A ₁ MRh ₁ ♂	8	96	24	64
150§	OM\NRh ₁	A ₁ \NRh ₂ ♀	1	64	2	96
151	OM\NRh ₁ Rh ₂	A ₁ \NRh ₁ Rh ₂ ♀	1	128	12	24
152§	OMRh ₁	A ₁ BM\NRh ₁ Rh ₂ ♂	24	24	16	24
153	OMRh ₁	A ₁ BM\NRh ₁ ♀	8	8	12	16
154	OM\NRh ₁ Rh	AMRh ₁ ♀	32	32	12	12
155§	OMRh ⁺	A ₁ BM\NRh ⁺ ♂	24	8	4	8
156	OM\NRh ₁	A ₁ NRh ₁ ♀	8	16	32	16
157	OMRh ⁺ Hr ⁺	A ₁ MRh ⁺ Hr ⁺ ♂	4	8	4	4
158	OM\Rh ⁺ Hr ⁺	A ₁ \Rh ⁺ Hr ⁺ ♀	48	24	12	12
159	OM\NRh ₁ Rh ₂ Hr ⁺	AMRh ₁ Hr ⁺ ♂	6	12	4	12
160	OM\NRh ₁ Hr ⁺	A ₁ BM\NRh ₁ Hr ⁺ ♀	4	16	4	16
161	OM\NRh ₁ Hr ⁺	A ₁ \NRh ₁ Hr ⁺ ♂	12	12	16	6
162	OMRh ₁ Hr ⁺	A ₁ MRh ₁ Hr ⁺ ♂	4	4	8	6
163§	OM\NRh	A ₁ BM\NRh ₁ ♀	24†	12	16+	24
164	OM\Rh ₁ Hr ⁺	A ₁ BM\NRh ₂ Hr ⁺ ♂	6†	24	12+	8
165	OM\NRh ₁	B\NRh ₁ ♂	16	24	8	16
166§	ONRh ₂	BM\NRh ₂ ♂	4	16	24	96
167	ONRh ₁ Hr ⁺	BM\NRh ₁ Rh ₂ Hr ⁺ ♂	4	6	8	6
168	ON\Rh ₁ Hr ⁺	BM\NRh ₁ Rh ₂ Hr ⁺ ♀	6	6	48	24
169	OM\NRh ₁ Hr ⁺	BM\NRh ₁ Hr ⁺ ♀	4	12	6	24
170	ON\Rh ₁ Hr ⁺	BM\NRh ₁ Hr ⁺ ♀	4	4	12	12
171	A ₁ BM\NRh ₁ Hr ⁺	B\NRh ⁺ Hr ⁺ ♂			8	12
172	A ₁ \NRh ₁ Hr ⁺	B\NRh ₁ Hr ⁺ ♂			8	32

TABLE 1—Continued

Case No	Mother	Child	Titer of Maternal Serum for			
			A ₁ Cells		B Cells	
			At Labor	1 Wk P P	At Labor	1 Wk P P
173§	A ₁ MNRh ⁻	A ₁ BNRh ⁻ σ [*]			3 [†]	3 [†]
174	A ₁ MRh ₀	A ₁ BMNRh ₀ ♀			3 [†]	12
175	A ₁ VRh ₁	A ₁ BMRh ⁻			6 [†]	4
176§	A ₁ MRh ₂ Hr ⁺	A ₁ BMRh ₂ Hr ⁺ σ [*]			12	16
177	A ₁ MNRh ₁ Hr ⁺	ABNRh ₁ Hr ⁻ σ [*]			1 [†]	4
178*§	A ₁ NRh ₀ Hr ⁺	ABNRh ₂ Hr ⁺ ♀			8	11
179*§	A ₁ MNRh ₂ Hr ⁺	ABMRh ₂ Hr ⁺ σ [*]			12	32
180§	BMNRh ⁺	AMRh ₁ Rh ₂ ♀	8	16		
181	BMRh ₁	A ₁ BMRh ₀ ♀	24	48		
182	BMRh ₂ Hr ⁺	A ₁ BNRh ₁ Hr ⁺ ♀	12	24		
183§	A ₁ MNRh ₁	A ₁ MNRh ⁻ σ [*]			16	16

P P = Post partum

* Negro families

† Titers determined two or more weeks ante partum no sample tested at delivery

‡ Twins

§ Primipara

σ No anti Rh agglutinins or blocking antibodies in maternal serum

|| Weak Rh agglutinin detected in maternal serum Infant clinically normal

** Infant stillborn

the two series is striking and indicates that the isoagglutinin titer probably does not change during a normal pregnancy. In a number of cases in which titrations were carried out earlier in pregnancy, the values obtained did not differ significantly from those obtained at the time of delivery.

A rise in the maternal isoagglutinin titer during the first week of the puerperium could reasonably be expected only in cases in which the fetus possessed an antigen A or B lacking in the mother. Accordingly, in table 3 the cases have been divided into two groups, namely, those in which the infant's red cells were compatible with the maternal serum with respect to the blood groups and those in which the infant's blood contained an A or B agglutino-gen absent from the maternal blood. In interpreting the results it is necessary to bear in mind that serologic titrations are extremely inaccurate in comparison with chemical titrations, and that therefore a difference in titer value of one or even two tubes is not unusual in the results obtained by careful workers using identical serum and test cells. Since the dilutions are prepared in geometric progression, any difference less than fourfold should therefore be discounted. These facts were borne in mind when we prepared table 3. As expected, significant rises in isoagglutinin titers occurred almost exclusively in the smaller group of cases in which the infant's red cells were incompatible with the maternal serum.

These results differ somewhat from those reported by Smith¹¹ and by Boorman and associates.¹² Smith observed a significant and specific rise in isoagglutinin titer in all but a small percentage of the cases in which the fetus possessed an agglutino-gen A or B lacking in the mother's blood. He also reported that in cases in which no rise in isoagglutinin titer occurred the infant proved to be a non-secretor.

The discrepancy between Smith's findings and our own is not as great as it appears to be at first sight. The majority of rises in titer reported by Smith amounted to only 2 or 3 dilutions, hence many of them may have been due to technical factors. Possibly parity of the mother may also have something to do with the difference between our results and his, because Smith's study was limited to primiparas. Moreover, we made only a single follow-up titration, and we may therefore have overlooked cases in which a rise in titer occurred before or after the day on which the test was made.

TABLE 2.—Comparison of Isoagglutinin Titers of Pregnant Women at Term with Isoagglutinin Titers of Normal Male Donors

Serum Titers	Normal Pregnant Women at Term*		Normal Male Donors†	
	Number of Group O and Group B Serums with Indicated Titer for A ₂ Cells	Number of Group O and Group A Serums with Indicated Titer for B Cells	Number of Group O and Group B Serums with Indicated Titer for A Cells	Number of Group O and Group A Serums with Indicated Titer for B cells
1	6	1	5	4
2	10	3	11	13
4	36	37	11	28
8	27	44	12	39
16	19	30	16	25
32	8	16	12	22
64	2	5	4	4
128	0	1	1	1
Totals	108	137	72	136

* Present study

† From Wiener and Moloney¹⁰

TABLE 3.—Rise in Maternal Isoagglutinin Titer After Delivery in Patients Without Toxemia

	Rise in Isoagglutinin Titers							Totals
	None or Less than 2 Tubes	2 ¹	2 ²	2 ⁴	2 ⁵	2 ⁶	2 ⁷	
Baby's red cells compatible with maternal serum	127*	2	0	0	0	0	0	129
Baby's red cells incompatible with maternal serum	29	5	0	1	0	1	1	37

Includes 4 cases in which titer dropped 2 tubes

As Smith pointed out, a rise in isoagglutinin titer could be caused by the passage of soluble group substances from the fetus across the placenta into the maternal circulation. In support of this idea are the following observations: 1. The intramuscular injection of small amounts of secretions containing group substances, e.g., as little as 0.2 cc. of autoclaved saliva, is sufficient to stimulate a marked rise in isoagglutinin titer.¹³ 2. It is well known⁶ that the amniotic fluid contains a high concentration of group substances in solution, hence it could be the source of the group substances stimulating the rise in the maternal isoagglutinin titer. The fact that whenever there is a rise in isoagglutinin titer this occurs about a week or two after labor suggests another possible source of the antigens that stimulate the rise in titer. It is possible that certain disturbances at the placental site occur during

TABLE 4—*Cases of Mothers with Toxemia*

Case No	Mother	Child	Titer of Maternal Serum for				Diagnosis	Clinical Findings
			A ₂ Cells		B Cells			
			At Labor	1 Wk P P	At Labor	1 Wk P P		
1*	OMNRh ⁻	OMNRh ♂	12	32	4	16	Preeclampsia	Edema
2*	OMNRh ₀	OMNRh ₁ ♂	16	8	8	8	Preeclampsia	Edema
3*	ONRh ₁ Rh	ONRh ₁ Rh ₂ ♂	2	2	2	2	Preeclampsia?	Hypertension
4*	OMRh ₁	OMRh ₁ ♀		12		8	Preeclampsia severe	Edema hypertension
5*†	OMRh ₁	OMNRh ₀ ♀	24	12	16	24	Eclampsia	Convulsions hypertension albuminuria
6*†	OMNRh ₂	OMNRh ₂	4	4	24	16	Preeclampsia	Edema hypertension albuminuria
7*	OMNRh ₁ Hr ⁺	OMNRh ₁ Hr ⁻	2	6	4	6	Eclampsia	
8	OMNRh ⁺	OMRh ⁺ ♀	16	16	32	32	Preeclampsia	Hypertension albuminuria
9*‡	ONRh ₁	OMNRh ₁ ♂	6	4	2	2	Preeclampsia	Hypertension albuminuria headache
10*†	OMNRh ₁	♂	8	16	8	12	Preeclampsia Premature separation of placenta	Edema hypertension
11*	A ₁ MNRh ₁	A ₁ MNRh ₁ ♂			2	2	Toxemia	
12*	A ₁ MNRh ₁	A ₁ MNRh ₁ ♂			4	8	Preeclampsia	Hypertension albuminuria
13*†	A ₂ MRh ₂	AMRh ₂ ♀			6	16	Preeclampsia	Edema hypertension
14*	A ₁ MNRh ₁ Hr ⁺	A ₁ MNRh ₁ Hr ⁺ ♂			8	12	Preeclampsia	Hypertension albuminuria
15*	A ₁ MNRh ₁ Hr ⁺	AMNRh ₁ Hr ⁺ ♂			6	8	Preeclampsia	Edema hypertension albuminuria
16*†	A ₁ NRh ₁	♂			4	4	Preeclampsia severe	Hypertension albuminuria
17	A ₁ 2MNRh ₁ Hr ⁻					50		
18	A ₁ MRh ⁻	OMRh ⁺ ♀			16	16	Preeclampsia	Edema hypertension
19*	A ₁ NRh ₁	OMNRh ₁ Hr ⁻ ♂			6	6	Eclampsia toxemia	Convulsion hypertension albuminuria
20*‡	A ₁ MNRh ₁	OMNRh ₁ ♂					Toxemia	
21*	BNRh ⁻	OMNRh ₁ ♀	4	128			Preeclampsia	Edema hypertension albuminuria
22 ‡	BMNRh ₀	OMNRh ₀ ♀	12	16			Eclampsia	Convulsions edema
23	BMNRh ₁ Rh ₂ Hr ⁺	OMRh ₂ Hr ⁺ ♀	8	12			Preeclampsia	Hypertension albuminuria
24*†‡	BNRh ₀ Hr ⁺	♂	4	2			Preeclampsia	Edema headaches
25*‡	A ₁ BNRh ₁ Hr ⁺	A ₁ BNRh ₁ Hr ⁺ ♂					Preeclampsia	Edema hypertension albuminuria
26*	A ₁ BMNRh ₁ Hr ⁻	{ BMNRh ₁ Hr ⁺ ♀ ♀ ♂					Premature separation of placenta	
27*	ONRh ₁	A ₁ MNRh ₁ ♂	16	384	16	96	Toxemia ²	Edema

TABLE 4—Continued

Case No	Mother	Child	Titer of Maternal Serum for				Diagnosis	Clinical Findings
			A ₂ Cells		B Cells			
			At Labor	1 Wk P P	At Labor	1 Wk P P		
28†	OMNRh	{ OMRh-♂ AMRh ₁ ♀	32	16	24	24		
29*	OMRh ₁ Hr ⁺	A ₁ MRh-Hr ⁺ ♀	8	12	8	12	Preeclampsia	Hypertension
30*	OMN Rh ₁	BMNRh ₁ Rh ♀	2	16	8	512	Preeclampsia	Edema hyper- tension albu- minuria
31†	OMRh ₂	BMNRh ₂ ♀	24	192	12	192	Preeclampsia	Hypertension albuminuria
32†	OMNRh ₂ Hr ⁺	BMNRh ₂ Hr ⁺ ♂	6	64	4	64	Preeclampsia	Edema hyper- tension albu- minuria head aches
33†	OMNRh ₂ Hr ⁺	BMNRh ₂ Hr ⁺ ♀	12	32	48	256	Preeclampsia	Hypertension albuminuria
34*	OMRh ₂ Hr ⁺	BMNRh ₁ Rh ₂ Hr ⁺ ♀	8	12	12	192	Preeclampsia	Hypertension albuminuria
35*	OMNRh ₁ Hr ⁻	BNRh ₁ Hr ⁺ ♀	6	6	24	24	Preeclampsia	Hypertension albuminuria

P P = Post partum

* Primipara

† Infant stillborn

‡ Negro

§ Child 6 months old when first seen because of Mediterranean anemia (familial erythroblastic anemia) Toxemia occurred during a preceding pregnancy

• Twins male twin died

|| Twins

labor and delivery, as a result of which villi may become detached and enter the maternal circulation. In support of this idea, it may be mentioned that autopsies on pregnant women frequently show the presence of Langhans giant cells or chorionic villi in the lung bed. The group substances in the tissues of the villi or the entrapped red cells could conceivably stimulate a rise in isoagglutinin titer in certain susceptible persons. In this connection, we have found that the best time to bleed patients for anti-Rh serum is a fortnight after delivery.

Mothers With Toxemia—In table 4 are listed the cases in which the mothers showed symptoms of toxemia, impending eclampsia or actual convulsions. The number is not large, but definite cases of this type are not frequent in the city of New York today.

The isoagglutinin titers of the mothers with toxemia at term did not differ significantly from those of the normal mothers.

In table 5 we have subdivided the cases of toxemic mothers into two groups (as in table 3 for normal mothers), according to the compatibility or incompatibility of the fetal red cells for the maternal serum. While there was such incompatibility in 37 of 166, or 22.3 per cent, of the infants of normal mothers, it was noted in 9 of 31, or 29 per cent, of the infants whose mothers had toxemia. Thus, the incidence of incompatibility is slightly higher in the presence of toxemia than when the mother is normal, but the difference is not statistically significant. Evi-

dently, A-B incompatibility producing isoimmunization of the mother plays no role in producing toxemia or at most accounts for only a small percentage of the cases. If an antigen-antibody reaction is the explanation for the toxemia, some other antigen than the agglutinogens A and B must be searched for.

TABLE 5—*Rise in Maternal Isoagglutinin Titer After Delivery in Patients With Toxemia*

	Rise in Isoagglutinin Titers (Tubes)							Totals
	None or Less than 2 Tubes	2	3	4	5	6	7	
Baby's red cells compatible with maternal serum	21	0	0	0	1	0	0	22
Baby's red cells incompatible with maternal serum	3	0	1	3	1	1	0	9

TABLE 6—*Comparison of Rh Factors in Normal Mothers and Their Children and in Mothers with Toxemia and Their Children (Tests with Anti-Rh₀ Serum)*

Rh Reaction of Mothers	Normal Mothers				Mothers with Toxemia			
	Infant		Totals		Infant		Totals	
	Rh -	Rh +	No	Per Cent	Rh -	Rh +	No	Per Cent
Neg	11	14*	25	13.7	3	3	6	13
Pos	16	142	158	86.3	1	25	26	81.5
Totals	27	156	183		4	28	32	
Per Cent	14.7	85.3		100.0	12.5	87.5		100.0

* In 1 case the mother had a very weak anti Rh₀ agglutinin but the infant was clinically normal (case 60 table 1).

TABLE 7—*Rh Types and Hr Factor in Mothers and Children**

Types of Mothers	Types of Children									Totals
	Neg	Rh ₁			Rh	Rh ₁ Rh	Rh ₀	Rh	Rh	
		Hr ⁺	Hr ⁺	Hr ⁻						
Neg	8	5	5	0	2	0	1	1	1	23
Rh ₁ ($\begin{matrix} Hr^{+} \\ Hr^{+} \\ Hr^{-} \end{matrix}$)	8	23	0	1	4	3	2	1	0	43
	6	0	19	7	0	2	2	0	0	36
	0	0	1	4	0	0	0	0	0	11
Rh	3	1	3	0	16	8	5	0	0	32
Rh ₁ Rh ₂	0	6	4	2	6	10	0	0	0	22
Rh ₀	1	2	2	0	3	0	13	0	0	21
Rh	1	0	0	1	0	0	1	2	0	5
Rh ⁺	0	0	0	0	0	0	0	0	1	1
Totals	24	34	40	15	31	23	24	4	2	103

* Includes 167 white and 36 Negro mothers.

† Test not done for Hr factor.

‡ All Rh₁Rh and Rh⁺ bloods tested for Hr were Hr⁺ as well as the bloods of types Rh₁, Rh₂, Rh₀ and Rh.

Of the 9 patients with toxemia who had infants belonging to an incompatible blood group, 6 were found to have a rise in isoagglutinin titer in the tests made one week after delivery. None of these patients had been treated by plasma trans-

fusion While the findings are suggestive, the series is too short to permit any conclusions

Rh Factor—In order to ascertain whether incompatibility with respect to the Rh factor plays any role in toxemia of pregnancy, our data have been analyzed in table 6 from the point of view of the reactions of the maternal and infant blood with standard Rh serums It will be seen that there is no significant difference between the normal mothers and those with toxemia, so that the Rh factor either is of no significance in toxemia or at most may account for only a very small percentage of cases

Inheritance of the Rh Blood Types and of the Hr Factor—Because of the small number of studies which have been made on the heredity of the Rh blood types and of the Hr factor, additional data which throw light on the hereditary mechanism are of interest For this reason, although this question is not related to the problem of toxemia, our findings on the Rh blood types and the Hr factor are summarized in table 7

With regard to the heredity of Rh blood types, under the six gene theory,^{14 15} and disregarding the rare intermediate genes and genes Rh_v and Rh_z , a type Rh_1Rh_2 mother cannot have a child of type Rh_0 or Rh negative, and mothers who are Rh negative or Rh_0 cannot have children of type Rh_1Rh_2 No exception to this rule was encountered in our series of patients

With regard to the heredity of the Hr factor, the genetic theory of Race and Taylor¹⁶ implies that Hr-negative mothers cannot have children of type Rh_2 , Rh'' or Rh_0 or Rh negative, while mothers of these four types cannot have Hr-negative children Again, no exception was encountered in our data

SUMMARY AND CONCLUSIONS

1 The titers of the isoagglutinins anti-A and anti-B in pregnant women at term do not differ significantly from the isoagglutinin titers in normal male adult blood donors This indicates that pregnancy itself does not ordinarily stimulate a rise in isoagglutinin titer

2 The frequency of incompatible blood groups in the infants of 31 mothers with toxemia of pregnancy was slightly higher (29 per cent) than that in a series of 166 infants of mothers without toxemia (22.3 per cent), but the difference was not statistically significant

3 Of 37 normal mothers whose infants had blood groups incompatible with theirs, 9 were found to have a significant rise in isoagglutinin titer one week post partum An appreciable rise in isoagglutinin titer occurred under similar circumstances in 6 of 9 mothers with toxemia The series are too small to permit definite conclusions regarding the significance of this in relation to toxemia However, there appears to be no doubt that under certain circumstances parturition can stimulate a rise in the maternal isoagglutinin titer

4 No correlation was found between the occurrence of Rh incompatibility and toxemia of pregnancy

5 The data obtained furnish additional evidence to support the theory of multiple allelic genes of heredity of the Rh blood types and also Race and Taylor's theory of inheritance of the Hr factor

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HEMATOLOGIC EFFECTS OF SPLENECTOMY IN STILL-CHAUFFARD-FELTY SYNDROME

A REPORT OF TWO CASES

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THE interrelationships of the blood, the bone marrow and the spleen remain among the major problems in hematology. Anemia, granulocytopenia and thrombocytopenia when present alone or occurring together are often associated with splenomegaly and a hyperplastic bone marrow. This triad, with variations and certain complicating factors, has been known for many years and has been noted in such diverse disorders as chronic tuberculous splenomegaly, hepatic cirrhosis, chronic arthritis, as well as in chronic malaria, leishmaniasis and other conditions with splenomegaly. Banti's syndrome, aplastic anemia, splenic anemia, Still's disease, Still-Chauffard's disease, Felty's syndrome, hypersplenism, hypoleukia splenica, myelosplenic syndrome, chronic agranulocytosis, and primary splenic neutropenia are some of the terms which have been more commonly used in describing these cases in the literature.

Confusion has been perpetuated by the lack of complete knowledge of the physiology and pathology of the spleen and its relationships to the bone marrow. In general, two concepts of the pathogenesis of this pancytopenic syndrome have arisen. Most European clinicians have adhered to the theory that a hormonal relationship exists between the spleen and marrow. According to this concept, a pathologically hypertrophied spleen produces a hormone which suppresses the maturation of blood cells in the marrow and their release to the blood. This hormone is thought to be produced by the reticulo-endothelial cells of the spleen and may act on all the cellular elements in the marrow at one time or be selective for the megakaryocytes, erythroid cells or granulocytes. The *normal* physiological maturation and release of blood cells in the marrow is perhaps controlled by a hormone which is also elaborated in the spleen or reticulo-endothelial system. This theory was apparently first publicized in 1912 by Isaac¹ in his interesting description of a case of splenic anemia and was further developed by Turk,² Frank,³ Lauda,⁴ Engelbreth-Holm,⁵ Schousboe,⁶ and Buchem.⁷ Naegeli⁸ was one of its champions and used the term *hypersplenism* with great effect in his description of cases in which the syndrome appeared. Dameshek^{9, 10, 10a} in this country has also used this term and has come out strongly for the hormonal concept of spleen-bone marrow relationships. Most American hematologists on the other hand have fostered the phagocytic hypothesis, in which the anemia, leukopenia and thrombocytopenia are thought to be due to the increased phagocytic activities of an enlarged spleen. The rate of destruction of blood cells is thought to exceed the rate of formation in the marrow, and cytopenia of a pure or combined type occurs.

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Wiseman and Doan¹¹ submit histopathologic evidence of phagocytosis in the spleen in support of the theory. They evidently accord little value to the concept of 'hormonal hypersplenism'. Doan and Wright¹² have recently re-emphasized their views in an article on Splenic Panhematopenia.

The occurrence of hypersplenism in association with chronic arthritis has been reported many times. Singer and Levy¹³ have carefully reviewed the literature which preceded Felty's report. Splenectomy has been used therapeutically in several cases. Steinberg¹⁴ in his investigation of the literature found that the results following splenectomy were not very satisfactory. Craven's case¹⁴ died 14 months after operation and Hanarahan and Miller's case survived only 18 months.¹⁵ Steinberg's patient became mentally depressed and weak following the splenectomy but was alive at the time he wrote his paper. Loeper, Andre and Patel¹⁶ described a case without leukopenia in which death from bronchopneumonia occurred four weeks after splenectomy. Dameshek's patient¹⁰ had a favorable hematologic response in addition to an increased resistance to infection. Of the two cases herein reported, the first is of particular interest because the patient has survived splenectomy five years and has maintained a greatly improved state of health.

Case No. 1 C. K. a white married female age 35 was admitted to the Medical Service on Oct. 7, 1940, with a history of arthritis, weakness, anemia and a weight loss of 20 pounds during the preceding three years. She had been well until four years prior to her admission. At that time she noted the rather abrupt onset of pain and stiffness in the joints of her hands and feet. It became necessary for her to discontinue her work as an elevator operator. Flexion deformity of the hands soon followed, and pain and stiffness in the shoulders, vertebral column, elbows and knees appeared during the succeeding months. The past history revealed that two years prior to admission she was observed at another hospital where a diagnosis of aplastic anemia was made. Upper respiratory infections, sinusitis, and stomatitis occurred frequently during these years and she stated that a fever of 100° and 101° was present most of the time. Just prior to her admission she was unable to get out of bed because of weakness and deformity. The past history and family history were irrelevant.

Physical Examination. The patient was a small, thin, pale woman. The weight was 42.5 kilo (93 lbs.), temperature 101°F, pulse 118 per minute. The joints, including those of the vertebral column, showed limitation of motion most marked in both elbows. Flexion deformities typical of the moderately advanced rheumatoid arthritic type were present in the hands and feet. The ankles were surrounded by soft tissue swelling. She was completely unable to walk. A chronic muco-purulent nasal discharge was present. The mouth was normal except for dental caries and hypertrophic pharyngitis. A loud systolic murmur was present over the entire precordium. The liver edge was palpable, smooth and non-tender, and a large non-tender spleen extended to the iliac crest and the mid-clavicular line. No palpable lymph nodes were evident. The skin was normal. A blood study on the day following admission revealed: R.B.C. 3.75 million, Hb. 9.5 Gm., W.B.C. 850, mature polymorphonuclears 0, band forms 7 per cent, lymphocytes 62 per cent, monocytes 31 per cent, hematocrit 32 per cent, M.C.V. 86 cu. microns, M.C.H. 25.3, M.C.H. Conc. 29.7 per cent, platelets 130,000 (normal 250,000 to 450,000), reticulocytes 4.5 per cent, 3-stippled erythrocytes per 100 leukocytes, erythrocyte fragility test (Sanford) normal, bleeding time (Duke) 5½ minutes, coagulation time 9½ minutes, capillary erythro-permeability test (Rumpel-Leed) negative, clot retraction time normal. A smear of marrow aspirated from the sternum on Oct. 9, 1940, showed very extensive hyperplasia of all myeloid cells with crowds of myelocytes and metamyelocytes and increased numbers of promyelocytes and myeloblasts. The erythroid cells were normoblastic and numerous. A gastric analysis showed achlorhydria. The icterus index was 9.6 and the Van den Bergh negative. Roentgenograms of the long bones and vertebral bodies were normal, i.e. there was no evidence of osteoporosis or joint destruction. The urine was negative and pyelography was normal. The electrocardiogram showed no abnormalities. Two blood cultures were negative. On Nov. 4, 1940, a splenic puncture was performed. A smear of the aspirated material revealed many lymphocytes and an apparently

increased number of reticulo-endothelial cells. There was no evidence of phagocytosis in several smears. Only a few granulocytes were seen. The culture was negative.

From Oct. 4, 1940, to Jan. 10, 1941, treatment consisted of blood transfusions, pentnucleotide, yellow bone marrow, ferrous sulfate and liver extract. There was no essential improvement in the patient's condition. The temperature varied between 99° and 103° F. During December 1940, an attack of acute maxillary sinusitis occurred. Splenectomy was finally decided upon and performed on January 10, 1941, without difficulty. The spleen was about the size of a football and weighed 1150 Gm. When the hemostats were removed from the pedicle, blood gushed to a height of about four cm. from the splenic vein and the enlarged organ collapsed to about two thirds of its original size. Epinephrine was not used during the course of the operation. Smears made from the splenic pulp were essentially the same as those made from the aspirated pulp obtained in November. Histologic examination of the spleen revealed a lattice work type of architecture with widely separated malpighian corpuscles. The capsule and trabeculae were thin.

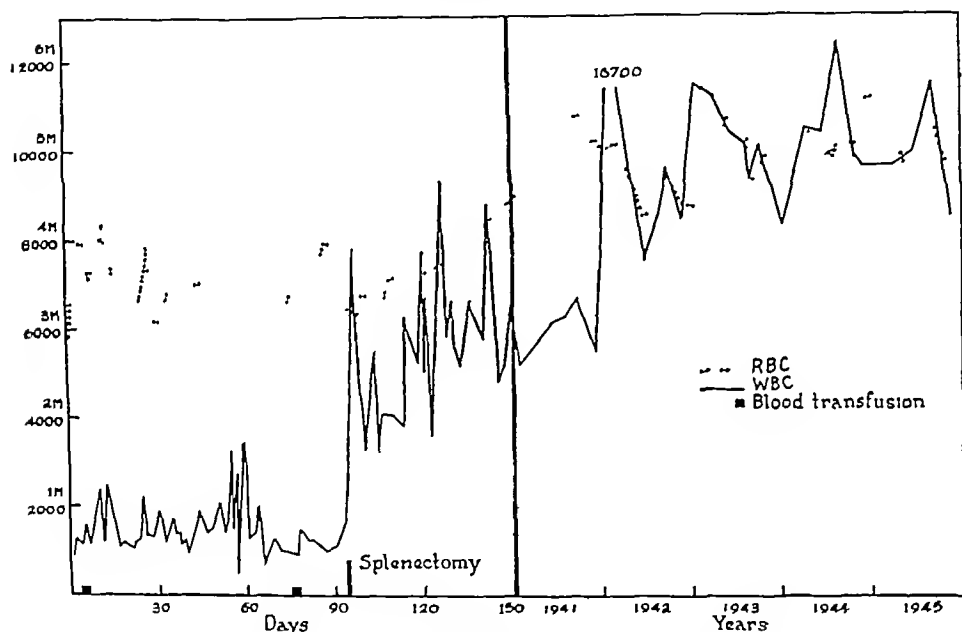


FIG. 1. LEUKOCYTE AND ERYTHROCYTE FLUCTUATION IN CASE 1

The red pulp of the spleen showed cleft like spaces with widely dilated sinusoids containing erythrocytes and many lymphocytes. Some of the sinusoids appeared to be plugged with large histiocytes. The Billroth cords were prominent and eosinophilic but showed only a very slight increase of connective tissue. Most of them were speckled with lymphocytes and histiocytes. The usual follicles were quite large and widely separated from each other. The lymphoid components of the peripheral portion of the follicles merged indistinctly with the adjacent pulp. Central arterioles were very eccentrically placed. The usual follicle had a very prominent germinal center composed of large cells with abundant cytoplasm. Minute particles of chromatin debris and blood pigment were visible and an occasional plasma cell was seen. There was no evidence of phagocytosis by histiocytes or other reticulo-endothelial cells observed in the sections.

The patient's postoperative course was uneventful except for a great increase in appetite. She was discharged from the hospital on March 9, 1941, able to walk haltingly without assistance. During the six months following the splenectomy she gained 13.2 kilo (29 lbs). Physiotherapy greatly increased the mobility of her joints, and by the end of 1941 she was able again to do her housework. During the last four years she has been able to climb stairs and ride on a bus without assistance. She does her own washing and ironing. The chronic sinusitis appears to have completely disappeared.

On Dec. 5, 1945, approximately five years later, she weighed 54.3 kilo (115 lbs). The blood sedi-

mentation rate at this time was 12 mm (Wintrobe corrected). Examination of the blood revealed R B C. 4.71 million Hb 13.5 Gm, M C V 95.7 cu microns, M C Hb 28.7%, M C Hb Conc 30 per cent W B C 8500, mature polymorphonuclears 35 per cent band forms 1. per cent eosinophiles 3 per cent lymphocytes 35 per cent, monocytes 15 per cent. There were 80 erythrocytes with Howell Jolly bodies per 100 leukocytes. Platelets were 276,000 per cu mm and reticulocytes 1.3 per cent. The arthritis had been reduced to a residual state of permanent flexion deformity of both hands and feet with slight limitation of motion in the left elbow. A graphic synopsis of the blood studies is presented in Fig. 1. A differential analysis of sternal marrow cells before and after splenectomy is found in table 1.

TABLE 1—*Differential Counts of Sternal Marrow Smears (Case 1)*

Splenectomy resulted in a relative increase in more mature forms, a diminution in cells of the erythroid fraction and a pronounced increase in lymphocytes.

	Before splenectomy		1 mo after splenectomy 2/11/41
	10/9/40	1/11/41	
	%	%	%
Myeloblasts	2.6	1.8	0.8
Promyelocytes	3.8	2.2	—
Myelocytes	26.2	17.4	10.8
Metamyelocytes, neutrophilic	17.4	20.0	19.4
Granulocytes neutrophilic band	8.6	5.8	12.8
Granulocytes neutrophilic segmented			1.2
Eosinophiles	1.0	1.6	1.8
Basophiles	0.2		0.2
Monoblasts			1.0
Monocytes		1.2	5.6
Lymphocytes	5.8	7.4	30.2
Plasma cells	0.6	0.2	3.6
Proerythroblasts	1.4	2.2	0.2
Basophilic normoblasts	6.2	12.4	0.2
Polychromatophilic normoblasts	10.8	13.8	1.0
Orthochromatic normoblasts	14.0	13.0	7.6
Megakaryocytes			0.2
Histiocytes		0.2	0.8
Unidentified blasts			0.8
Cells in mitosis	1.4	0.8	0.2

Case No. 2 E E, a white single female, age 49, was admitted to the Medical Service on Aug. 5, 1941 complaining of inability to care for herself. She was a Christian Scientist and would make no statements regarding pain. Four years prior to admission she began to notice stiffness in her knees, ankles and wrists. Two years later the fingers of both hands became stiff and flexed so that she was unable to continue her occupation as an artist. Since then she had gradually become bedridden and helpless and was brought to the hospital for nursing care.

Physical Examination. Weight 54.5 kilo (120 lbs.) temperature 99.6 pulse 80 blood pressure 94/60. The patient was unable to sit up without assistance. Moderately severe flexion deformity of all joints of the extremities was present. The hands had the typical appearance of advanced rheumatoid arthritis with glossy smooth skin and ankylosis. A loud systolic murmur was heard over the entire precordium. The spleen and liver edges were both palpated about 3 cm. below the costal margin. These organs were firm.

and smooth. The remainder of the examination was insignificant. There were no enlarged lymph nodes and the skin was not pigmented. Examination of the blood shortly after admission revealed WBC 950, segmented polymorphs 1 per cent, band forms 6 per cent, eosinophiles 2 per cent, basophiles 1 per cent, lymphocytes 83 per cent, monocytes 7 per cent. RBC 3.59 million, Hb 10.25 Gm, hematocrit 38 per cent, MCV 108 cu microns, MCHb 29.77, MCHb Conc 27 per cent. There were 50 stippled erythrocytes per 100 leukocytes. There were 282,000 platelets and the icterus index was 9.7. Free hydrochloric acid was present in the gastric secretion. Blood culture was negative. Roentgenograms of the hands and knees revealed almost complete loss of joint space as well as mild hypertrophic arthritis. Osteoporosis was present in the bones of the wrists, hands and vertebral column. The urine was normal. Splenic puncture was not attempted because of the relatively small size of the palpable spleen. Sternal marrow aspiration revealed a slightly hyperplastic marrow with a left shift in both the myeloid and erythroid cells (table 2). A low grade fever of 99° to 101° was present almost daily. This patient, because

TABLE 2.—*Differential Count (500 Cells) of a Sternal Marrow Smear before Splenectomy (Case 2)*

	%
Myeloblasts	0.0
Promyelocytes	9.2
Myelocytes, neutrophilic	7.2
Myelocytes, eosinophilic	2.4
Metamyelocytes, neutrophilic	8.4
Metamyelocytes, eosinophilic	5.4
Granulocytes, neutrophilic band	5.2
Granulocytes, neutrophilic segmented	1.2
Eosinophiles	8.4
Basophiles	
Monocytes	0.6
Lymphocytes	12.6
Plasma cells	3.8
Proerythroblasts	0.4
Basophilic normoblasts	3.2
Polychromatophilic normoblasts	23.6
Orthochromatic normoblasts	6.8
Megakaryocytes	0.2
Unidentified cells	1.2

of her religion, was for some time not interested in accepting treatment. After a period of depression she became anxious to submit to any type of therapy. A splenectomy was recommended with the understanding that her advanced arthritis might not be changed but that the blood picture might approach the normal.

On Nov. 10, 1941, splenectomy was performed. Blood was taken from the splenic artery and splenic vein before their ligation. The spleen and two small accessory spleens were removed. The enlarged spleen was adherent in part to its bed. A liver biopsy specimen was removed. Smears of splenic pulp were prepared immediately. They showed as many as 8 to 20 nucleated cells per oil immersion field. Approximately 90 per cent of these cells were lymphocytes. Plasma cells, eosinophiles and a few band and segmented granulocytes were also present. Platelets were always aggregated in groups of about 20 to 80 platelets. Cells readily identified as monocytes were occasionally seen. Many of the reticulo-endothelial cells were very similar to monocytes. An occasional blast form with 3 to 5 nucleoli was observed. Typical, large reticulo-endothelial cells were present singly and in groups of 2 to 5 throughout the smears. They accounted for less than half of 1 per cent of all the cells. About half of the adult reticulo-endothelial cells which were observed revealed evidence of phagocytosis and digestion of other cells. The ingested cells were pyknotic and were undergoing lysis as evidenced by weak and altered staining and loss of structural outline. The remnants of ingested band and segmented granulocytes, erythrocytes, platelets

and lymphocytes were seen. Frequently the type of ingested cell could not be determined and often several different cells were found included in one reticulo-endothelial cell. Section of the splenic tissue revealed prominent follicles and fibrosis of the red pulp. The trabeculae were thin and the Billroth cords thickened and acellular in many places. The sinusoids were outlined clearly in some areas but were very narrow and compressed in others. Plasma cells and lymphocytes were prominent throughout the pulp. The malpighian corpuscles were prominent and had a sharp lymphocytic periphery. The germinal centers were unduly large and active. Many of the cells were pyknotic and mitosis was seen. The sections show no evidence of phagocytosis by reticulo-endothelial cells. The spleen weighed 525 Gm.

The patient had a stormy postoperative course which was precipitated on the fourth day by a sudden hemorrhage from the incision. She had received 100 mg. of heparin during the day because of the rising platelet count. The wound was slow in healing and on the twenty-second day she developed ascites and ankle edema. The temperature fluctuated between 100° and 101°. On the thirty-seventh day it was necessary to perform an abdominal paracentesis, releasing 7600 cc. of cloudy fluid. The patient expired on the next day. The changes in the blood picture following splenectomy are tabulated in table 3.

TABLE 3—Changes in the Blood following Splenectomy (Case 2)

Date	W B C (per cu mm)	Band forms	Mature polys	Eos.	Lymph	Mono	Baso	Platelets (per cu. mm)	Howell Jolly bodies per 100 W B C
	%	%	%	%	%	%	%		
11/7/41	1200	1		8	76	16	2	122,000	0
11/10/41 (before removal of spleen. Patient under general anesthesia)	1500	28	2	2	58	10	0	129,000	6
3 hours after splenectomy	1800	67	10	0	14	9	0	220,000	1
1st post op day	8400	not done						169,000	not ob- served
2nd post-op day	6500	47	32	2	9	10	0	473,000	2
3rd post-op day	5500	37	49	2	8	4	0	385,000	6
4th post-op day	4550	28	52	0	16	4	0	296,000	38
5th post-op day	5000	28	45	2	17	8	0	424,000	20

An autopsy was performed and the following pertinent findings reported. There were contractures of the thighs and legs and both legs were edematous. One and a half liters of thick, cloudy fibrino-purulent fluid were found in the abdominal cavity. Heavy fibrous adhesions were present between the liver and the diaphragm and between the transverse colon and the abdominal wall. The esophagus was dilated and contained large dilated tortuous vessels. Both lungs were attached to the thoracic wall by old fibrous adhesions. The tracheo-bronchial lymph nodes were enlarged, fused, deeply pigmented and soft. The heart was normal except for slight atheromatosis in the proximal portion of the ascending aorta. The liver weighed 2030 Gm. Its surface was slightly granular, and when cut, the lobules were seen to be surrounded by small white depressions. Microscopic examination of the liver tissue obtained during the splenectomy and at autopsy revealed marked fatty degeneration in the peripheral portions of the lobules and hyperplasia of the periportal connective tissue with lymphocytic, eosinophilic and plasma cell infiltration in these areas. The bile canaliculi were dilated. The greatly thickened gallbladder was distended and contained three mixed cholesterol calculi, each about 1½ centimeters in diameter. The portal vein was completely thrombosed at its point of entrance into the liver. Purulent degeneration was present in the center of the thrombus which was firmly adherent to the lining of the vein. Thrombosis was present in all the intrahepatic branches of the portal vein which could be seen. The splenic vein pedicle was completely thrombosed, and the mesenteric veins supplying the large and the distal portions of the small intestine were almost completely thrombosed. The stomach, intestine, kidney, adrenals, pelvic organs and lymph nodes were essentially normal. Microscopic examination of an abdominal lymph node

revealed indistinct germinal centers composed of small, deeply stained lymphocytes. Sinusoids distended with erythrocytes, lymphocytes and some plasma cells were present.

DISCUSSION

It is quite evident that splenectomy was followed by an increase of the granulocytes in the peripheral blood in both cases. This occurred almost immediately in case 2 but more gradually in case 1. Both patients presented monocytosis before and after splenectomy. Post-splenectomy thrombocytosis was present in each, and case 1 was characterized by a gradual and complete recovery from the anemia.

If the enlarged spleen were phagocytizing cells from the blood stream one would expect a difference in cell counts between blood in the splenic artery and that in the splenic vein. Passage through the spleen should perceptibly change

TABLE 4—*Comparison between Blood from the Splenic Artery and Splenic Vein (Case 2)*

The blood was obtained immediately before the spleen was excised

	Splenic artery	Splenic vein
R. B. C. (millions)	4.29	4.12
Hemoglobin (grams)	13.0	13.0
Hematocrit (%)	38	43
M. C. V. (μ^3)	90	104
M. C. Hb. ($\gamma\gamma$)	30	31
M. C. Hb. Conc. (%)	34	30
W. B. C.	11,700	2,600
Myelocytes (%)	1	0
Metamyelocytes, neutrophilic (%)	1	0
Granulocytes, neutrophilic band (%)	9	8
Granulocytes, neutrophilic segmented (%)	1	3
Eosinophiles (%)	6	0
Basophiles (%)	0	0
Monocytes (%)	8	14
Lymphocytes (%)	71	74
Plasma cells (%)	3	1

the character of the blood. In case 2, blood was taken from the splenic artery and the splenic vein just prior to their ligation before excision of the spleen. The leukocyte count in the splenic arterial blood was 11,700, while the count in the splenic venous blood was similar to what had been observed in the peripheral blood prior to the splenectomy, namely 2,600 per cu. mm. Examination of the blood smears verified the difference in the concentration of the leukocytes. Doan has recently reported a similar observation.¹⁶ Table 4 expresses a comparison between the splenic arterial and venous blood. Examination of the differential counts reveals similarity except for a greater left shift and eosinophilia in the arterial blood and a higher percentage of monocytes in the venous blood. If leukocytic phagocytosis occurs in the spleen it would be expected, on the basis of this observation, that it would affect evenly all cell types, with a slight preference for the younger cells. The evidence in this case, that more leukocytes enter than leave the spleen, tends to favor the phagocytic rather than the hormonal theory.

The adherents of the hormonal theory base their belief on several observations, of which the most important is the apparent immaturity and maturation arrest which is seen in marrow smears. The great numbers of erythroblasts and myelocytic cells and, at times, megakaryocytes which are seen crowded in the smears and the paucity of mature or even relatively mature cells lead the observer to a conclusion that maturation arrest is present. If mature cells are present in the marrow and diminished in the blood it is concluded that there is faulty emission of cells from the marrow. Histological evidence of cellular phagocytosis in the spleen is seldom sufficient to explain the hyperplasia in the marrow. A more convincing argument depends upon the observation that after splenectomy the number of blood cells rises slowly over a period of days and some times weeks, according to a pattern of escape from maturation arrest. If the spleen were merely phagocytizing, one would expect a very abrupt increase in cells in the blood after splenectomy. Then, too, the phagocytic theory fails to explain the presence of old segmented granulocytes and the absence of metamyelocytes and myelocytes when peripheral leukopenia is present. The opposite would be expected. It is difficult to believe that, with a leukocyte count of 850 in the presence of a very hyperplastic marrow, metamyelocytes would be absent from the peripheral blood if the spleen were removing great numbers of leukocytes. A marrow thus strained to the limit of its productive capacity would pour immature cells into the blood in great profusion and one would expect them to predominate in the blood residue.

The experiments of Bock and Frenzel¹⁷ and of Jombres¹⁸ may eventually help explain the phenomenon. The former investigators ligated the splenic vein and the gastric coronary veins in rabbits in such a way that the venous outflow from the spleen passed into the esophageal veins and the superior vena cava by way of the left gastro-epiploic vein and a collateral circulation in the stomach. This double ligation prevented blood which left the spleen from passing through the liver. The rabbits developed the hypersplenic syndrome. Jombres performed essentially the same experiment and observed that the femoral marrow became hyperplastic several weeks after ligation. It is possible that an essential factor in the mechanism of hypersplenism is a retardation of blood flow through the spleen. Bock and Frenzel and Jombres adhere strictly to the hormonal theory of hypersplenic suppression of the marrow and propose that the liver enters into the hormonal mechanism inasmuch as their ligations effectively prevent splenic venous blood from passing through the liver.

The answer to the problem of hypersplenism may eventually be found in a combination of the hormonal and phagocytic theories. The evidence now available supports, in part, both sides. Thus hypersplenism may well have a complex origin.

SUMMARY AND CONCLUSION

Two cases of Still-Chauffard-Felty syndrome treated by splenectomy are presented. One case has survived five years with complete hematologic restitution and definite improvement in the arthritis. The second case expired six weeks after

splenectomy Autopsy revealed portal vein thrombosis (pylephlebitis) Both cases exhibited the blood manifestations associated with hypersplenism. In both cases splenectomy resulted in a return of the peripheral blood picture to relative normality

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EDITORIAL

THE TREATMENT OF POLYCYTHEMIA

THE treatment of polycythemia has passed through several successive stages since Osler first used benzol to reduce the excessive blood cell formation. Phenylhydrazine, a hemolytic poison, was introduced by Morawitz following its experimental use by J. H. Pratt. Although this new drug achieved wide popularity its unpredictable nature and the severe hemolytic crises which often ensued made its use relatively hazardous. In some instances transfusions actually became necessary—a rather embarrassing situation. Other deleterious effects were on the circulation, the kidneys, and the liver. What is more, all the elements including iron for enhancing further red cell formation, were retained within the body. For these reasons, the systematic use of multiple venesections to reduce the red cell mass and blood volume and induce a state of iron deficiency seemed far more physiological. In our own experience, the removal of 500 cc. of blood twice weekly for two or five weeks—depending upon the initial hematocrit and hemoglobin levels—has proved a satisfactory method. To maintain somewhat longer the resulting state of iron deficiency, it has been our practice to keep the patient on a diet low in iron. Red cell formation under these circumstances is only partially reduced, but hemoglobin and hematocrit levels remain low for periods of six to eighteen months, during which time the patient may be completely asymptomatic. Red cell levels during this induced remission gradually rise, so that the red cell count as an index of therapy is of little value. The best index is the hematocrit value although the hemoglobin concentration alone may be used since this correlates fairly closely with the hematocrit level. With this method of therapy, patients go along for many years with very little more difficulty than do others in the older age group in which polycythemia occurs.

The use of x-ray therapy has in our hands proved ineffective, either by heavy dosage to localized areas or by spray or generalized therapy. We have furthermore hesitated to use high voltage x-ray in these essentially normal individuals because of the possible dangers of radiation malignancy or leukemia. The more recent introduction of radio-phosphorus, as discussed by Erf in this issue, makes therapy a good deal easier and is ordinarily productive of an excellent remission. Here too we have hesitated to use a potentially dangerous radioactive material in an individual with a relatively long life span. One naturally wonders whether the acute leukemic states which have occurred in some cases are due to the drug or are associated with the polycythemia. The data at present are insufficient to permit statistical analysis of this point. The results of careful follow-up studies in cases treated with P^{32} will be awaited with interest.

WILLIAM DAMESHEK, M.D.

ABSTRACTS

HEMOGLOBINEMIA AND HEMOGLOBINURIA

JOSEPH F. ROSS, M.D.

HEMOGLOBIN PRECIPITATION IN RENAL TUBULES. A STUDY OF ITS CAUSES AND EFFECTS. *Yuile, C. L., Gold M. A. and Hinds E. G. J. Exper. Med.* 82: 361-74, 1945

Yuile and his collaborators report a continuation of their well conceived and carefully controlled studies of the mechanism of hemoglobin excretion and its effects on the kidney. They point out that in their experience injections of large amounts of purified solutions of hemoglobin do not produce hemoglobin casts or tubular blockage in normal rabbits regardless of whether the urine is acid or alkaline, an observation confirmed by several other investigators.

However, injection of hemoglobin solutions into rabbits with pre-existing tubular damage (produced by clamping the renal artery for a period of 15 to 25 minutes or by injecting sodium tartrate solution) resulted in definite impairment of kidney function. In rabbits with alkaline urine this renal dysfunction was transient and did not result in death. In animals with acid urine extreme renal failure, uremia, and death occurred. Hemoglobin casts were few in number and soon disappeared from the tubules in the animals with alkaline urine but were numerous, persistent and indicative of tubular blockage in the aciduric rabbits.

It was of interest that in instances of extreme pre-existing tubular damage (produced by sodium tartrate) hemoglobin casts were not found even in animals with very acid urine. In such instances hemoglobin probably was not excreted by the kidney and thus did not enter the tubular lumina.

The authors conclude that the ultimate outcome in any given instance of hemoglobinuria is dependent upon a fine balance between the degree of renal injury and the level of hemoglobinuria, as well as upon the pH of the urine.

RENAL DAMAGE FROM FERROHEME PIGMENTS: MYOGLOBIN, HEMOGLOBIN, HEMATIN. *Corcoran A. C., and Page, I. H. Texas Reports on Biol. & Med.* 3: 528-44, 1945

In an attempt to elucidate the etiology of the renal failure occurring in cases of crush injury and transfusion reaction, investigations were made of renal function following injections of myoglobin, metmyoglobin, hemoglobin and hematin into aciduric dogs. Renal function was evaluated from studies of the clearances of diodrast and inulin and of the tubular secretory capacity for diodrast. Unfortunately the hemoglobin solutions used were simply solutions of lysed dog red blood cells and undoubtedly contained possible toxic substances other than hemoglobin. Solutions of purified pigments would have been more desirable. Control studies on dogs with alkaline urine are not reported.

Renal function was impaired in all experiments regardless of whether hemoglobin, myoglobin or metmyoglobin was injected and consisted in an immediate decrease in the tubular secretory capacity followed by a reduction in the renal plasma flow and in the glomerular filtration rate. Impaired function in some instances was greater 48 hours after the injection than during the period of myoglobinuria and persisted in mild degree for the two week period of the study. The renal injury was not severe enough to produce uremia or death and anatomical studies are not reported.

Hematin was extremely toxic when injected intravenously and produced death from shock when given in a dose of 32.6 mg. per Kg. It apparently produced intense efferent arteriolar vasoconstriction in the kidney, glomerular damage and a marked decrease in renal plasma flow and tubular secretion. Uremia developed in an animal which received the injection of a smaller dose.

The authors attribute the renal injury to (1) obstruction of the tubules by pigment (although they did not demonstrate such obstruction), (2) ingestion of the pigment by tubular cells with resulting impairment of tubular secretory activity (no evidence was presented that ingestion of pigment interferes with tubular function) and (3) cytotoxic distal tubular activity of intratubularly liberated hematin.

BLOOD SUBSTITUTES

EUGENE L. LOZNER, COMMANDER, MC(S) USNR

THE USE OF A MODIFIED GLOBIN FROM HUMAN ERYTHROCYTES IN HYPOPROTEINEMIAS *Strumia M W Blake, A D, Reider H C, and Chornock, F W* Am J M Sc 211 51-61 1946

This represents a follow up on the preliminary report previously abstracted (Blood 1 89 JAN 1946) by Strumia and associates, on the intravenous administration of a preparation of globin from human erythrocytes. In the present paper seven patients with various types of hypoproteinemias have been treated with globin intravenously. It is apparent that globin is a useful substitute for plasma in this regard. This statement derives chiefly from the fact that globin does not seem to be excreted rapidly in the case with protein hydrolysates, amino acids or degraded gelatin. However, nitrogen balance and plasma protein studies of much longer duration are required before it can be stated unequivocally that globin is well utilized for the synthesis of body protein. The mere existence of a positive nitrogen balance when an intravenous protein is being administered is not sufficient evidence to prove this point. It indicates only that the protein is not rapidly excreted. The concepts of the meaning of a nitrogen balance, that date from the days when ingestion was the only source of nitrogen, will now have to be revised with the introduction of nitrogenous material intravenously. As was stated previously, Strumia's work is quite promising because human erythrocytes are a much richer protein source than plasma. They are at present discarded in large amounts. Consequently the preparation of any useful therapeutic serum from this source would indeed be praiseworthy.

CHEMICAL, CLINICAL AND IMMUNOLOGICAL STUDIES ON THE PRODUCTS OF HUMAN PLASMA FRACTIONATION XXX THE USE OF SALT POOR CONCENTRATED HUMAN SERUM ALBUMIN SOLUTION IN THE TREATMENT OF CHRONIC BRIGHT'S DISEASE *Thorn, G W, Armstrong, S H, Jr, Dickerson, V C, Woodruff, L M and Taylor, F H L* J Clin Investigation 24 802-28, 1945

Thorn and his associates report on the treatment of seven patients in various stages of chronic nephritis with salt poor human serum albumin at a dosage of 50 grams a day for varying periods. Significant diuresis was achieved only in those patients with nephrotic anasarca. This diuresis was stated by Thorn and associates to be unlike that previously recorded as following the administration of serum plasma or acacia and unlike the spontaneous diuresis of the nephrotic state. The latter two diureses persist after the period of therapy. With albumin on the other hand the diuresis was stated to proceed at a constant rate during the period of administration and to stop at the end of it. Careful scrutiny of the seven patients presented by Thorn reveals that only two of them had anasarca and in these two on one occasion the diuresis persisted for at least several days after the albumin was discontinued and in the other the diuresis stopped during the administration of albumin. It is evident therefore that more data are needed before it can be satisfactorily concluded that the diuresis following albumin proceeds at a constant rate during the period of administration only and stops at the end of it. Thorn and associates point out that albumin administration in the presence of severe hypertension and nitrogen retention in the absence of edema appears contraindicated owing to the danger of overloading the cardiovascular system. They also point out their study does not indicate that the diuresis, when induced by albumin, results in any change in the natural history of the disease process. No deleterious changes in renal function as a result of the large doses of albumin were observed. It is obvious that much more work is needed to decide whether the beneficial effects produced by human albumin in nephrotic anasarca are great enough to justify the cost of 50 grams a day over long periods of time.

HEMOSTASIS AND THE HEMORRHAGIC DISEASES

L. M. TOCANTINS, M.D.

NOUVELLES ÉTUDES SUR LE HÉMOPHILE RÔLE DES ALBUMINES PLASMATIQUES DANS LA FORMATION DE LA THROMBINE *Fessly R* Helvet med acta 11 177-88 1944

The author studied the role of hemophilic plasma albumin in the formation of thrombin. Thrombin and other plasma fractions were obtained from platelet free plasma by precipitations with ammonium sulfate.

sulfate, acidification with dilute sulfuric acid, followed by dialysis. The albumin fraction isolated from normal plasma added to a solution of normal globulin does not exert any delaying effect on the speed of thrombin formation. The albumin fraction isolated from hemophilic plasma added to a solution of normal globulin delays by several hours the time of the appearance of thrombin. This inhibiting property of the albumin fraction is destroyed by heat (67°C) is manifested before the formation of thrombin but has no effect on thrombin after it is formed.

In view of the above findings the author has modified his previous stand (Helvet med acta 8 823, 1941) that in hemophilia the fundamental defect is a deficiency of the activator of prothrombin contained in the viscous protein fraction of the plasma associated with the globulins. The stability of hemophilic plasma is now attributed by the author to a neutralization of the clot accelerating globulins by inhibitors in the albumin fraction. The inhibiting effect of albumin may be overcome by the prothrombin fraction of the plasma, but not by the fraction containing only the prothrombin activator. These last observations may also be interpreted as showing that, though in the presence of enough prothrombin, clotting will eventually take place in spite of the albumin inhibitor, the clot accelerating effect of prothrombin free plasma thromboplastin is reduced or overcome by hemophilic plasma albumins.

STABILITE DE LA PROTHROMBINE DANS LE SANG CONSERVE Lavergne, G. H., and Lavergne Poindessault, B.
Compt rend Soc de biol 136 445-46, 1942.

The authors have attempted to account for the striking lack of agreement between prothrombin determinations by the one-stage and two-stage methods when carried out on aged citrated (or oxalated) plasma. Whereas by the one stage method 24 hour old plasma kept at 0°C will show a diminution of prothrombin of about 50 per cent, no significant decrease is observed in eight days, under the same conditions when prothrombin is estimated by the two-stage method. One important difference between the two technics is that in the two-stage method a purified fibrinogen solution is used, while in the one stage method the fibrinogen of the aged plasma itself is employed. The disagreement between the two technics seems due to the fact that in aged plasma, denaturing of fibrinogen is rapid and this affects its rate of transformation to fibrin by thrombin. The authors conclude that (a) the one stage method should be used only in fresh plasma, (b) it is not suitable for measuring prothrombin of aged plasma because the denatured fibrinogen falsifies the result (indeed, a naturally resistant fibrinogen may produce the same effect) (c) prothrombin is stable in citrated blood for eight days at 0°C and such blood is as suitable for transfusions as fresh blood in so far as prothrombin is concerned.

The authors' demonstration that fibrinogen was the deficient factor was brought about by mixing prothrombin free $\text{Al}(\text{OH})_3$ adsorbed plasma with aged plasma. Such mixtures lead to a normal prothrombin time (allowing for the factor of dilution) by the one stage method. The explanation seems to be that normal reacting fibrinogen is supplied by the prothrombin free adsorbed plasma, while prothrombin as such is supplied in normal amounts by the aged plasma. Variations in the conversion rate of fibrinogen seem therefore like variations in the prothrombin conversion rate to contribute to the lack of agreement occasionally observed between results obtained by the two methods. Other implications of these experiments are apparent. According to Quick the loss of prothrombin activity in aged plasma is due to destruction of component A of the prothrombin complex while in $\text{Al}(\text{OH})_3$ and dicoumarinized plasmas component B is absent. If the observations of the French workers are confirmed they would tend to indicate that component A is simply fibrinogen undenatured by standing.

HEMATOPOIETIC TISSUES

O. P. JONES, PH D

THE MIDBODIES IN HUMAN ERYTHROBLASTS Schwarz, E. Anat Rec 92 363-70 1945

During an investigation of the divisional capacity of maturing human erythroblasts it was found that preparations stained for centrosomes and centrioles were also suitable for demonstrating midbodies. The present paper reports observations made on dividing megaloblasts in two marrows obtained from pernicious anemia patients during relapse. Midbodies first make their appearance in late anaphase before the first signs of cellular constriction but in the path of the future divisional furrow. They are however finally excluded from the daughter cells which is evidence opposing the view that they are related to

centriole formation. These studies show that megaloblasts do not differ from other animal cells with respect to their midbody formation. This is of great interest, since megaloblasts are abnormal in several other respects.

A QUANTITATIVE STUDY OF TISSUE FLUID—LYMPH CELLULAR RATIOS *Allen, L. Anat. Rec. 91, 279-81, 1945*

By studying the cellular content of tissue fluid (peritoneal) and peripheral lymph from the diaphragmatic plexus, several problems pertaining to the lymphocyte have been attacked. It was found that there are approximately five times as many leukocytes in tissue fluid as in lymph. This may be due to the fact that the lymphocyte is the most common cell in tissue fluid. The evidence presented indicates that motile and nonmotile bodies have the same absorptive ratios, which supports the author's contention that the mechanics of lymphatic absorption is intercellular rather than intracellular. Suspensions of marrow cells pass through the endothelium in about the same proportion to their concentration. Assuming that the properties of lymphatic endothelium and the reticulo-endothelium are identical in all respects, the author suggests that these experiments offer a uniform theory for the delivery of blood cells to the circulation. However, it should be pointed out that most histologic and hematologic evidence has not supported this view.

FRAGMENTATION OF AMPHIBIAN ERYTHROCYTES IN THE ULTRACENTRIFUGE *Beams, H. W., and King, R. L. J. Morphol. 77, 63-69, 1945*

The ultracentrifugation of rat erythrocytes demonstrated that they contain three substances differing in specific gravity and perhaps physicochemically (Beams and Hines, *Anat. Rec.* 90, 155, 1944). In the present paper, similar experiments have been made with Necturus and frog erythrocytes. When ultracentrifugal forces from 20,000 to 400,000 times gravity were employed for periods of from 5 to 30 minutes, the amphibian erythrocytes demonstrated a remarkable degree of elasticity by returning from a distorted shape to a normal one. Hemolysis did not occur when erythrocytes were fragmented, indicating that the cells have more structure than a fluid bladder. Fragments of amphibian erythrocytes tended to round up instead of returning to or maintaining a flattened shape. This seems to indicate that ultracentrifugation alters their viscosity and membrane properties. Ultracentrifugation of amphibian erythrocytes produced a stratification not unlike that reported for the mammalian corpuscle.

THE FUNCTION OF LEUCOCYTES IN THE GROWTH AND REGRESSION OF THE EGG OF *TRITURUS VIRIDESCENS* *Liebman, E. Am. J. Anat. 77, 273-91, 1945*

Functions of the several leukocytes have been determined from time to time by subjecting them to various experimental conditions. The present article is unique in that it was possible to study three different leukocytes while they participated in more or less related phenomena—the growth and regression of amphibian eggs. Lymphocytes were attracted to the healthy egg, engulfed and finally assimilated. Hence, lymphocytes add to the substance of the growing egg and in this respect act as trophocytes. Neutrophils invade the egg shortly after it shows the first signs of degeneration and break up the yolk into fragments. After phagocytizing much of this material, the neutrophils undergo a necrosis and finally die. The remaining traces of the corpus arreticum are removed by follicle cells and macrophages (transformed lymphocytes). Eosinophils were the only leukocyte found in the small yolkless atretic ova. The distinct and deeply eosinophilic specific granules of these cells coalesce into a homogeneous mass after they have invaded the egg. This homogeneous material is finally liberated into the cytoplasm and aids in the formation of channels. These studies indicate that the catabolic activities of the neutrophil and eosinophil are quite dissimilar.

FUNCTIONAL ALTERATIONS IN LYMPHOID TISSUE INDUCED BY ADRENAL CORTICAL SECRETION *Dunst, T. F., and White, A. Am. J. Anat. 77, 81-106, 1945*

In recent years the work of the Yale group and others concerning the cellular source of antibodies has attracted considerable attention (*J. A. M. A.* 128, 1232 [Aug. 25] 1945). The present paper is the result of an extension of these studies to the field of hormonal control of the release of serum globulin from lymphocytes. Adrenotropic hormone, adrenal cortical extract, compounds E and F, corticosterone, desoxycorticosterone acetate, prolactin and human serum gamma globulin were administered to groups

of normal and adrenalectomized mice and normal rabbits. Marked changes were observed in the lymphoid tissue of these animals as early as one hour following the injection of a single dose of adrenotropic hormone or adrenal cortical extracts. Histological changes were divided into three types: degenerative changes, repair changes and recovery changes. These histological alterations were correlated with certain physiological roles of the lymphocytes. Evidence is presented which indicates that the action of adrenotropic hormone on lymphoid tissue is mediated by the adrenal cortex. Since several unrelated stimuli may increase pituitary adrenal cortical secretion, these probably account for the so-called "accidental involution" of lymphoid tissue. The mechanism by which lymphocytes contribute serum gamma globulin to the blood in normal animals and antibody protein in immunized animals is a dissolution of lymphoid tissue.

LYMPHATIC TISSUE AND REGRESSIVE STRUCTURE, WITH PARTICULAR REFERENCE TO DEGENERATION OF GLANDS. *Kingsbury, B. F.* *Am. J. Anat.* 77: 159-187, 1945.

The presence of lymphatic tissue and accumulations of lymphocytes in the major salivary glands has been recognized for a long time. However, little or no attention has been given to similar structures within the minor salivary glands. The present paper deals with a study of 33 series of the larynx of the cat. These were fixed in Bouin's fluid, Zenker's fluid, Helly's or Maximow's Zenker formol and stained with either hematoxylin and eosin, Mallory's connective tissue stain or methylene azure-phloxine neutral stain. The particular glands studied were: (1) mucous glands of the root of the tongue, (2) epiglottic and subepiglottic laryngeal glands, (3) the tracheo-laryngeal glands of the lower larynx, and in the soft palate (4) the palatine (salivary glands), (5) the nasopharyngeal glands and finally (6) the glands of the laryngo-pharynx. The small and usually atypical lymphatic nodules sometimes had clear areas which seemed to be equivalent to proliferative centers. Cells associated with the lymphatic tissue were small lymphocytes (lymphocytoid cells), elastic cells and large elastic cells. To the reviewer it seems unfortunate that the latter cells were so designated, since it implies that leukemic cells are normally present in this tissue. Two types of polymorphonuclear cells were encountered. The first was found within duct lumen and seemed to represent derivatives of lymphocytoid cells which were to degenerate ultimately. The second type of polymorphonuclear cell had a nuclear pattern quite similar to that of the blood neutrophil but lacked cytoplasmic granules. Since these were usually found in areas of the soft palate where there was marked degeneration, it is thought that these cells arose locally in response to some inflammatory process.

MITOTIC DIVISION AND DEGENERATION OF LYMPHOCYTES WITHIN THE CELLS OF INTESTINAL EPITHELIUM IN THE MOUSE. *Andrew, W., and Andrew, N. V.* *Anat. Rec.* 93: 251-78, 1945.

The presence of lymphocytes in the gastrointestinal epithelium has been subjected to many and diverse interpretations as to their exact morphology, location and function. For example, they have been considered to be normal and degenerate, intercellular and intracellular, mitotic and nonmitotic, and finally migrating toward the lumen and not migrating to the lumen. Material for the present study was obtained from 10 mice of the C57 Black strain. Longitudinal sections were made through the pyloric portion of the stomach and duodenum. An examination of 200 epithelial cells in the crypts of Lieberkuhn and on the villi of each of the 10 animals revealed that most of the lymphocytes are intracellular. These lymphocytes undergo degenerative changes as they pass from the basal layer toward the lumen. Mitotic figures, both normal and abnormal, are limited almost exclusively to the crypts of Lieberkuhn. The appearance and behavior of lymphocytes in the epithelial cells of the gastrointestinal tract are similar to those seen in secondary lymphatic nodules. It has been hypothesized that the activity of these lymphocytes may be in the nature of a defense reaction.

A METHOD FOR STUDYING NUMERICAL AND TOPOGRAPHIC PROBLEMS IN THE WHOLE FEMORAL MARROW OF RATS AND GUINEA PIGS WITH THE USE OF UNDECALCIFIED SECTIONS. *Mayer, E., and Ruzicka, A. Q.* *Anat. Rec.* 93: 213-31, 1945.

If hematological investigations require the determination of the presence or absence of cell types, then the dry smear imprint or section technique applied to small samples may be used advantageously. But if it is desired to obtain information relative to the distribution of cell types throughout the entire marrow, this problem is attended with many technical difficulties, especially when small laboratory

animals are used. The authors obviated these difficulties by using suitably grooved wooden blocks to hold the femora while removing the epiphyses and corticis with a Moto Tool prior to fixation. After fixation more of the corticis is ground off and the pencil of marrow removed. Small cheesecloth bags are used to carry the marrow pencils through various phases of paraffin embedding. This new technique for obtaining marrow pencils may be employed to extend knowledge regarding the variation of megakaryocytes under certain conditions, the equilibrium between hematopoietic tissue, fatty tissue and sinuses, and the delivery of myeloid cells into the circulating blood.

THE MOTION OF THE MIGRATING CELLS IN TISSUE CULTURES OF LYMPH NODES. De Bruyn, P. P. H. *Ann Rec* 93: 295-315, 1945

Studies on the manner of locomotion of blood and tissue cells in vitro led Rich, Wintrobe and Lewis (Bull. Johns Hopkins Hosp. 65: 291, 1939) to conclude that myeloblasts, lymphoblasts and mononuclear phagocytes were unrelated. The present study was undertaken to consider the relationship between lymphocytes and monocytes in tissue culture, since the above observations were in disagreement with the unitarian theory of hematopoiesis. De Bruyn cultured abdominal lymph nodes from rabbits and recorded the locomotion of cells with time lapse photographs on reversible film at the rate of 60 per minute. Sixteen series of 25 to 35 cultures each were studied after 3, 8, 16, 33 and 52 hours incubation. In the early cultures lymphocytes migrate in two phases. The first is an active locomotory phase in which the cell has an anterior pseudopodial area and a posterior tail-like projection. Such cells are said to be polarized. The second phase is a nonlocomotory phase in which the pseudopodial area and tail are withdrawn—or depolarized. Although such a state has been called a rest period, small pseudopodia are continually arising. When lymphocytes hypertrophy they tend to shorten their locomotion phases. Macrophages in the older cultures were continually in the depolarized phase. A genetic relationship was established between these cells on the basis of gradual transitional stages in the mode of migration between the typical lymphocyte and the hypertrophied lymphocyte, and between the latter and macrophages.

THE SPLEEN

S. ESTREN, M.D.

SPLENIC INJURY. Rhame, H. E. U. S. Nav. M. Bull. 45: 342, 1945

This report concerns a patient who had been splenectomized for traumatic rupture of the spleen six years before he came under the supervision of the author. An abdominal operation for an unrelated cause revealed a tumor in the midjejunal serosa, and several similar tumors along the greater curvature of the stomach, the lower margin of the transverse colon, and in the greater omentum. Microscopically all of these tumors were hyperplastic hemolymph nodes which had taken on the characteristics of splenic tissue (except for absence of the trabeculae). One large mass was definitely an accessory spleen.

According to Rhame, these changes resulted from the removal of the spleen in a young adult and were an attempt to restore the function of the spleen by hypertrophy of existing hemolymph nodes and accessory splenic tissue. It parallels occasional reports of the presence of multiple splenic nodules throughout the peritoneal cavity after trauma to the abdominal wall.

PRIMARY SPLENIC NEUTROPENIA. Rogers, H. M. and Hall, B. E. Arch. Int. Med. 75: 192-96, 1945

PRIMARY SPLENIC NEUTROPENIA WITH REPORT OF A CASE. Salzer, M., Ransohoff, J. L., and Blatt, H. Ann. Int. Med. 22: 271-73, 1945

SPLENIC NEUTROPENIA. REPORT OF A CASE WITH SPLENECTOMY. Langston, W. White, O. A., and Altlin, J. D., Jr. Ann. Int. Med. 23: 667-72, 1945

These three articles report one case each of chronic idiopathic neutropenia cured by splenectomy. The symptoms included listlessness, weakness, repeated infection, and splenomegaly. The cases are further examples of the entity first described by Wiseman and Doan in 1939, which is being recognized with increasing frequency. The case of Rogers and Hall showed, in addition to a neutropenia, moderate anemia and thrombocytopenia, and clinical and biopsy evidence of hepatitis. It illustrates therefore a pancytopenia or panhematopenia rather than a pure neutropenia. It is possible that the hepatitis was associated with chronic splenic dysfunction since liver function became normal after splenectomy.

No definite statements as to pathogenesis appear in these reports. The bone marrow was consistently normal. In the first report, the spleen showed no evidence for segregation or phagocytosis of white cells. This was also true in the second case. In case 3, on the other hand, granulocytes were numerous in the splenic capillaries and phagocytosis of polymorphonuclears was noted. The question of excessive destruction of granulocytes by the spleen in contrast to their nonliberation from the bone marrow as a result of suppressing effect of the spleen, is yet to be settled.

DU MÉCANISME DE L'ANÉMIE AU COURS DES ANÉMIES SPLENIQUES DE L'ADULTE SPLENOMÉGALIES HÉMO-
LYTIQUES ET SPLENOMÉGALIES MYÉLOPÉNIQUES (MECHANISM OF SPLENIC ANEMIA IN THE ADULT HEMO-
LYTIC AND MARROW INHIBITING SPLENOMÉGALIES) *Abrams, P., de Gaudart d'Allaines, F. and Dugas*
J. Sang 16 213-18 1944

This is one of the first available articles on hematology from France in the past few years. The authors defend the concept of a splenic anemia as an entity parallel to splenic pancytopenia and to idiopathic thrombocytopenic purpura (splenic thrombocytopenia). They separate from the wastebasket splenic anemia, a few distinctive cases of nonhemolytic anemia associated with splenomegaly and cured by splenectomy. They distinguish this entity from hemolytic anemias, Banti's disease, etc. One case is presented in detail and several others are mentioned.

The basis for the disease, according to the writers, is splenic inhibition of the bone marrow resulting in a disturbance not only in the production of cells in the marrow, but also in their liberation into the blood stream. This inhibitory action may evidence itself only on one particular type of cell (giving neutropenia, anemia or thrombocytopenia) or on two or more types of cells. When all three types are affected, pancytopenia results. Splenectomy cures all these conditions by eliminating the inhibitory factor.

ENDOCRINE FUNCTION OF THE SPLEEN AND ITS PARTICIPATION IN THE PITUITARY-ADRENAL RESPONSE TO STRESS *Ungar, Georges* *Endocrinology* 37 329-40, 1945

The author describes a crystalline substance which he has isolated from the spleen of guinea pigs and which he designates splenin. In guinea pigs, this substance was found to have three actions: (1) it reduced bleeding time, (2) it increased capillary resistance, (3) it inhibited the release of histamine from blood cells. Studies were done in normal, hypophysectomized, adrenalectomized and splenectomized guinea pigs. In each type of animal, the bleeding time was studied after injury, hemorrhage, starvation, and the use of certain drugs. Splenectomized guinea-pigs showed increased bleeding time after injury, this could be returned to normal by use of splenin. A pituitary-adrenal reaction to stress is described, which was inhibited by splenectomy, and returned to normal by splenin. Extracts of pituitary, adrenal and spleen had similar actions in this regard, and splenectomy inhibited the action of each type of extract. Other organ-extracts (liver, kidney, nodes, etc.) had no effect.

The author considers his experiments to prove that the spleen behaves as an endocrine organ and participates with other endocrine organs, notably the pituitary and adrenal, in a combined reaction to certain stimuli. The author does not reveal the method by which he extracted splenin and thus makes impossible duplication of his results. The isolation of a crystalline hormone from the spleen would be of great importance in further evaluation of splenic physiology, but further work is necessary before this goal is reached. The value of a specific substance that would reduce bleeding time is also obvious. If verified, this work would be one of the first direct proofs of the endocrine nature of the spleen.

SEVERE TYPE OF HEREDITARY ANEMIA WITH ELLIPTOCYTOSIS. INTERESTING SEQUENCE OF SPLENECTOMY *Cooley, T. B.* *Am. J. M. Sc.* 209 561-68, 1945

Three types of heredo-familial anemia which do not respond to iron are well known. These are congenital spherocytic hemolytic anemia, Mediterranean anemia, and sickle-cell anemia. The latter two are hypochromic anemias, yet they do not respond to iron therapy. Cooley describes in the present report a new form of heredo-familial anemia which is hypochromic in type, which does not respond to iron therapy, and whose characteristic cell is an elliptocyte. Nineteen of 29 boys in the mother's line of descent had severe anemia; 16 of these died, one recovered spontaneously, and two are the patients studied by Cooley. No girls were affected. The systemic nature of the disorder is suggested by the stunting of one boy's growth, and the progressive character of the anemia. There was no evidence for a he-

myolytic process Splenectomy was done because of the lack of effect of other modes of therapy, and resulted in slow steady improvement of moderate degree

Cooley suggests that his patients represent a new group of anemias due either to a defect in the red blood cell, or to an inability to utilize more than some maximum amount of iron This same defect has been postulated to explain Mediterranean (target cell) and sickle-cell diseases and it seems reasonable to group these new cases with them despite the absence of a hemolytic component The effect of splenectomy was definite although not dramatic The further course of the patient should be of interest

ANAEMIA ASSOCIATED WITH UNIDENTIFIED ERYTHROCYTIC INCLUSIONS AFTER SPLENECTOMY *Pappalardo*

A M Thompson, W P, Parker, D D and Smith, K E Quart J Med 54: 75-100 1945

This report concerns three cases of hypochromic anemia which did not respond to iron or iron-folate medication Splenectomy was performed because of progression of the disease The first two cases died after splenectomy, the third case showed no essential hematologic or clinical change after splenectomy In all three cases post splenectomy blood smears showed small iron staining coccoid or bacilliform bodies in the red cells, which resembled Bartoocella bodies Cultures and animal inoculations, however failed to demonstrate Bartoocella organisms It was concluded that the bodies were iron-containing granules of obscure significance, resembling similar bodies described by Grünberg in certain animals

Refractory hypochromic anemias form a heterogeneous group of disorders from which various entities are being segregated Mediterranean disease and sickle-cell disease are two such entities Cooley's patients (described above) may represent a third one and the three cases of the present report may represent still another The intra-erythrocyte bodies are probably of significance, although similar ones have occurred after splenectomy for other diseases (Grünberg)

RUPTURE OF SPLEEN IN INFECTIOUS MONONUCLEOSIS *Davis, J S, MacFie, W, Wright, M and Allie F*

Lancet 2: 72-73, 1945

In a three-month period in 1943, the authors saw 18 cases of infectious mononucleosis in one arm hospital of which one was complicated by rupture of the spleen Rupture occurred on the 14th day of the patient's illness, and was characterized by a sudden sharp pain at the left upper abdomen and left shoulder tip, the appearance of surgical shock, and the disappearance of the previously palpable spleen A vertical longitudinal split of the splenic capsule was found at operation, and there were two other tears in the splenic pulp Splenectomy was curative

SPONTANEOUS RUPTURE OF A MALARIAL SPLEEN *Ross, S E, and Gaynor, J S* J A M A 127: 75⁶ 1945

A soldier aged 28 had had a single attack of vivax malaria overseas Eleven months later, while in the United States, he had a sudden chill and fever plus acute pain in the left shoulder and left upper abdomen There was no shock Operation revealed a ruptured spleen with an abdomen full of fresh and clotted blood Splenectomy was curative Nine days later, plasmodium vivax was recovered from the blood during another chill

SPONTANEOUS RUPTURE OF THE SPLEEN IN MALARIA FATAL CASE *Kellner A, Hochstein, E, and Teller A* J B J A M A 128: 1227-29 1945

A soldier aged 28, had his first attack of malaria two weeks after his return to the United States from the South Pacific, where he had taken atabrine daily and not had malaria There were several more attacks in the following ten months At this time another attack occurred One day after its onset the patient suddenly developed chill vomiting numbness of the legs, abdominal pain blindness, surgical shock and died within 2½ hours Autopsy revealed multiple lacerations of a very soft spleen and an abdomen full of blood

The question of slight trauma is considered by the authors The patient had a good deal of vomiting and straining during his attacks It is suggested that abdominal palpation and excessive coughing, vomiting, and straining may perhaps be related to rupture of a spleen which is already softened by malaria or, perhaps other inflammation

SPONTANEOUS RUPTURE OF A NORMAL SPLEEN *Duby, H* New England J Med 233 207-08, 1945

A young woman was suddenly seized with upper abdominal pain, inability to take a deep breath, and fainting. Examination resulted in the diagnosis of active intraperitoneal bleeding, probably secondary to ectopic pregnancy. The only finding at exploration was a soft spleen with a rent near the upper pole. Splenectomy was curative. The spleen weighed 340 grams and showed multiple lacerations, but no cause for the rupture was demonstrable.

Rupture of a normal spleen is a rare occurrence. The fact that this spleen was soft may have contributed to its rupture. Specific diagnosis is not to be expected in such cases, but splenic rupture may have to be considered in intraperitoneal bleeding from unknown cause.

RUPTURED ANEURYSM OF SPLENIC ARTERY *Martin, F E* U S Nav M Bull 44 152-53, 1945

The authors report a Navy officer who complained of sudden abdominal pain which was followed by shock, distention, and death. Autopsy revealed a perforated aneurysm of the splenic artery just proximal to the gastro-epiploic branch. No clue as to etiology could be obtained. The spleen itself was normal. The vascular system was otherwise normal. There were no signs of lues.

PRIMARY SPLENIC NEOPLASMS *Bostusck, W L* Am J Path 21 1143-65, 1945

The author reviews the 157 recorded cases of primary splenic neoplasms in the literature, and adds seven of his own. He recognizes seven types: (1) angioma, both hemangioma and lymphangioma, (2) lymphoma, (3) reticulo-endothelial tumors including endothelioma and reticulum-cell sarcoma, (4) embryonic inclusions, including epithelial cysts, dermoids, and mesothelial cysts, (5) fibrosarcoma, (6) leiomyosarcoma, (7) neurosarcoma. The latter tumor is theoretical only, as no examples have yet been reported. The listing is in descending order of frequency.

Of the seven new cases, the chief complaint of the patients was the presence of an abdominal mass. In one case there were signs of a ruptured viscus. In two cases, the splenic tumor was an incidental finding at autopsy.

CALCIFIED CYST OF THE SPLEEN *Jamison, E M, and Smith, O F* U S Nav M Bull 45 537-41, 1945

This report concerns an 18 year old naval student with a calcified cyst of the spleen. His symptoms were of three months' duration and included abdominal pain, paresthesiae of the fingers, and pain in the left lower chest. Examination showed limited excursion of the left diaphragm, and moderate left upper quadrant tenderness. A calcified mass in the left upper abdomen was discovered on x-ray and was shown not to be connected with the gastrointestinal tract or the kidneys. A splenectomy was done, and the spleen showed an irregular, partially calcified cyst in the upper pole. The patient's symptoms were completely relieved.

Only 7 calcified cysts of the spleen had been reported, 1943, and only 152 splenic cysts of all types by 1941. As a rule, cysts produce no symptoms. Occasionally, the patient finds a mass in the left upper quadrant. The x-ray demonstration of annular calcification is practically pathognomonic, although rarely an aneurysm of the splenic artery may be partially calcified.

QUISTE HIDATICO DEL BAZO ABIERTO EN BRONQUIOS (HYDATID CYST OF SPLEEN WITH RUPTURE INTO A BRONCHUS) *Uncabalo, D, Mainetti, J M, and Cuculicchio, C* Revista medica de hosp Ital de la Plata (Argentina) 1 173-75, 1944

This is another report of a relatively rare splenic disease with splenomegaly. A young woman with anorexia, malaise, weight loss, cough, chills, and fever was found to have signs of consolidation at the left lower chest and a large spleen. Examination of the sputum revealed tubercle bacilli, but the hooks of *tenia echinococcus* were easily found. An abdominal operation was performed at which a large multiloculated cyst was found replacing the spleen. It was adherent to the left diaphragm and had apparently ruptured into the left chest. Formalization and marsupialization were performed with excellent recovery. At a nonrelated abdominal operation almost three years later, the only sign of a spleen was a fibrous thickening at the left subphrenic region. The chest was normal.

In all these reports of splenic tumors, relatively few laboratory data are given by which one might ascertain their effect on the blood elements.

NEWS AND VIEWS

MISCELLANEOUS

Under the auspices of the Office of Public Assistance and Hygiene and of the Institute of Hemotherapy of Córdoba, Argentina, the first course of instruction in hemoplasmothrapy ever held in Argentina was given between August 9 and 14, 1945. The organization of the course was under the direction of Arturo R. Pezzi, head of the Institute of Hemotherapy. Lectures and demonstrations were given in the Maternity Hospital and in the Children's Hospital. The Argentine Society of Hematology and Transfusion Therapy was organized. The Council of the Society will be made up of Drs. Jenaro García Oliver, Arturo R. Pezzi, Alberto Battaglia, Luis Agotz, Robertson, Augusto Romero Alvarez, Miguel Angel Etcheverry, Serafin F. Villalobos and Carlos F. Gatt.

The Hematology Research Foundation of Chicago announces that the Helen Schuman Finnerman Fund will be used for the hospitalization of special patients in connection with the treatment of blood diseases. A second fund, called the Ruth Reader Fellowship in Hematology, will be used to aid graduate students. The Foundation, organized in 1944, has a medical advisory council of seven members: Dr. Raphael Isaacs, Michael Reese Hospital, Chicago; Dr. Louis R. Limarzi, assistant professor of medicine, University of Illinois College of Medicine; Dr. Andrew C. Ivy, professor of physiology, Northwestern University Medical School; Dr. Ludvig Hektoen, Chicago Tumor Institute; Dr. Anton J. Carlson, professor emeritus of physiology, University of Chicago School of Medicine; Dr. Italo F. Volini, formerly dean of Loyola University School of Medicine; Dr. Otto Saphir, pathologist, Michael Reese Hospital and University of Illinois. The Foundation has 757 members.

Dr. William H. Cole, director, Rutgers University Research Council, reports that Rutgers University, nine pharmaceutical manufacturers, and the Army Quartermaster Corps are pooling their knowledge and resources in an extensive cooperative research program which is making basic scientific studies of the properties and therapeutic values of protein hydrolysates and amino acids. Studies on the use of proteins, protein hydrolysates, and amino acid mixtures in normal and hypoproteinemic dogs were started to answer basic questions for a better understanding of protein metabolism.

The Blood Transfusion Association of New York will resume grants in 1946 for research in the field of blood and blood substitutes in relation to transfusion. Those interested in obtaining such grants should write, giving full information.

concerning their project, to Chairman, Research Grants Committee, 2 W 106th Street, New York City

The Nebraska State Health Department began doing Rh factor tests on obstetric patients last year without charge

Ground was broken by Mayor William F Devin of Seattle for the new home of the King County Central Blood Bank The Blood Bank recently completed its first anniversary

PERSONALIA

In connection with the development of the Medical College of Alabama in Birmingham, there is being established a hematological center for the study of blood diseases and research in hematology This activity will be under the direction of Dr Roy R Kracke, who is Dean of the School, assisted by Dr William H Riser, Jr, Associate Professor of Clinical Pathology

Dr Carl V Moore gave the Christmas Seminar at the King County Hospital in Seattle on the 27th, 28th, and 29th of December, 1945 In that series of lectures, he spoke on (1) Radioactive Phosphorus in the Treatment of Leukemia and Allied Disorders, (2) Pathogenesis and Therapy of the Hypochromic Anemias, (3) Pathogenesis and Treatment of the Hemorrhagic Diatheses, and (4) Recent Observations on the Antianemic Effect of Folic Acid

Dr O P Jones received a personal letter from Dr J Groen, Head of the Second Medical Department, Wilhelmina-Gasthuis, Amsterdam-W, Holland in which he stated that since they have been isolated for 5 years, there is an urgent need for medical literature Reprints, used books, back numbers of journals will be invaluable Any American journal that arrives here circulates like a treasure and is devoured like manna from heaven! , he writes

Dr Joseph F Ross has received a promotion to the rank of Associate Professor of Medicine at the Boston University School of Medicine He is completing work on the problem of preserving blood and erythrocytes in blood banks, which he has been carrying on for the past two and a half years under a contract with the Office of Scientific Research and Development

Dr Eugene L Lozner, U S N R, was recently promoted to Commander and appointed Assistant Research Executive of the Naval Medical Research Institute He continues as Head of the Hematology Department

Lectures during 1946 at the Academy of Medicine of Cincinnati included one by Dr Bruce K Wiseman, Columbus, January 8, The Spleen and Blood Formation, and one by Dr Charles A Janeway, Boston, April 2, Studies on Clinical Uses of Plasma Protein Fractions

The State University of Iowa College of Medicine, Iowa City, conducted a course in the operation of blood transfusions and on related subjects February 4 to March 2. The course was under the direction of Drs Elmer L. DeGowin, assistant professor of internal medicine, and Robert C. Hardin, associate in internal medicine.

Col. Loren D. Moore, M.C., U.S. Army, retired, who recently became assistant director of the division of biologic laboratories of the Massachusetts Department of Health, will be in direct charge of the new civilian blood service program which has been inaugurated by the State Department.

BOOK REVIEWS

Die Thrombozyten des menschlichen Blutes und ihre Beziehung zum Gerinnungs- und Thromboserorgan By PROF DR MED A FONIO AND DR MED J SCHWENDER Hans Huber, Bern, Switzerland 1942 Distributed in U S A by Grune & Stratton, Inc New York Pp 130, S3 00

This monograph on platelets is a welcome addition to the constantly increasing number of fundamental studies on the morphology and physiology of the blood cells. The behavior of the blood platelets is studied by an ingenious new technic which utilizes photomicrographs taken with a dark field microscope. Platelets were studied in plasma suspensions prevented from clotting by chemical means (magnesium sulfate, sodium citrate) and in plasma which was prevented from clotting by freezing immediately after withdrawal of the blood.

The platelets assumed changes in form within the first 12 to 24 hours: they were oval or round immediately after puncture, then they rapidly became irritation forms (within four hours), at which stage pseudopodia appeared with end bulbs which attached the platelets to foreign objects such as glass slides. This stage was followed by swelling and disintegration. Some of the platelets went into a rest form before disintegration. The process of degeneration included segmentation of pseudopodia, separation of ring forms and disintegration of the complete platelet. Agglutination heaps resulted from the adherence of several platelets by means of interlocking pseudopodia and bodies.

In cooled plasma the changes were similar to those in plasma rendered noncoagulable by sodium citrate or magnesium sulfate. Here, however, it was possible to follow the process of coagulation more readily. Thus as the plasma became warmed, masses of platelets were readily detected, and the sticking of the heaps to the glass slides, the appearance of fibrin needles in the process of clot retraction could be ascertained. Similar events were found in the capillaries of the frog in *vivo* after diathermic injury.

The monograph is excellently conceived and beautifully illustrated with numerous dark field photomicrographs depicting in great detail the various platelet changes. It is unreservedly recommended to all interested in platelet physiology and the study of coagulation and thrombosis.

Haemoglobin Levels in Great Britain in 1943 (with Observations upon Serum Protein Levels) By THE COMMITTEE ON HAEMOGLOBIN SURVEYS MEDICAL RESEARCH COUNCIL His Majesty's Stationery Office London 1945 Pp 128 Price 2s

In 1943 a special committee was appointed by the Medical Research Council of Great Britain to survey the hemoglobin levels in large social groups of the population in an attempt to obtain evidence as to the nutritional state of the people of Great Britain after four years of war. The results of the investigation are presented in this monograph.

After theoretical and technical considerations, the authors present details on various groups in the population. Briefly some of the conclusions are as follows. The incidence of low hemoglobin levels in the population in 1943 was not so great as in previous studies, but was still high in certain specialized groups notably young children, pregnant women and people in low income brackets. The mean hemoglobin for men was only slightly below the mean hemoglobin value considered satisfactory before the onset of the war. The mean hemoglobin for women was 8 to 10 per cent lower than that for men at most ages. There was some difference between married and unmarried women, attributable to the reduction in hemoglobin found to accompany repeated pregnancies. As the number of pregnancies increased in any age group before the menopause, a small but consistent decline in hemoglobin level was noted. Serious anemia on the other hand, was rare in all groups, although the incidence of anemia in general was disproportionately high at ages 2 to 5 and some 7½ per cent of men and women in all groups had hemoglobin levels below 90 and 80 per cent respectively.

In addition to the factual data, certain theoretical considerations are of interest. The monograph includes a discussion of physiological factors which influence hemoglobin levels together with an excel-

lent chapter by Dr R. G. Macfarlane on the errors of hemoglobin estimation by the Haldan-Gowat method (a comparator method using carboxyhemoglobin)

The monograph is well produced and excellently fitted to the purpose for which it was intended. It will presumably serve too as a base line for postwar investigation of nutrition and thereby allow a partial evaluation of the effect of war upon a people's erythropoietic system.

THE PRESENT STATUS OF FOLIC ACID

B₃ L. JOE BERRY, PH D, AND TOM D SPIES, M D

WITH the recent demonstrations¹⁻⁵ of the effectiveness of folic acid (synthetic *L. casei* factor) in the treatment of pernicious anemia, nutritional macrocytic anemia, and of sprue, it was considered sufficiently important to review the literature dealing with the various aspects of the work with this promising material. A review of this subject is timely not because the complete story of its discovery and identity can be told but rather because its clinical significance seems to urge the telling of its development as far as our present knowledge permits.

Folic acid is one of the newest members of the vitamin B complex. Like other better known members of this group, its discovery came from various laboratories with widely divergent approaches. In fact it is impossible to state with assurance that the divergent beginnings have all converged on the same substance, but at least in part they appear to have done so at this time. Thus, for the sake of clarity, we shall attempt to develop in the following pages each apparently unrelated group of studies individually until they begin to overlap. We shall then attempt to show how these studies have become integrated. No special effort to maintain proper chronology will be made.

I. GROWTH FACTORS FOR *Lactobacillus casei* AND *Streptococcus lactis* R

In 1940, Snell and Peterson⁶ published the tenth paper of a series entitled "Growth Factors for Bacteria" in which they revealed that certain lactic acid bacteria required extracts of plants or animals for growth. The basal medium was composed of purified hydrolyzed casein supplemented with riboflavin, pantothenic acid, niacin, pyridoxine, and tryptophan. *Lactobacillus casei* of Freudenreich was used as the test organism (Bergey classifies this bacterium as *Lactobacillus helveticus*). Yeast extract (Bacto) and solubilized liver fraction were both rich sources of the essential growth factor. It was found that a norite eluate factor was the most active fraction from these sources. Methods of concentrating the active principle were investigated and tests were made to determine some of its chemical properties. A compound of basic character was found which was labile to oxidation and was precipitated by many common basic precipitants. It showed some properties in common with naturally occurring purines. The following year Hutchings, Bohonos, and Peterson⁷ described a procedure used in further purifying the eluate factor from solubilized liver. Their material was not precipitated by well known basic precipitants so that the material was definitely less basic than that originally suggested. In fact studies with electrodialysis and esterification demonstrated

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clearly the acidic nature of the active principle. The destruction with nitrous acid and inactivation on acetylation or benzylation suggested the presence of an amino group. The fact that the activity did not depend on the phosphorus content of the preparation led to the conclusion that a nucleotide was not an active part of the compound.

Earlier in the same year Stokstad⁸ had isolated a factor necessary for the growth of *L. casei* from solubilized liver by adsorption on norite and elution with 0.5 N ammonium hydroxide in 70 per cent methanol. Further purification consisted of fractional precipitation of the manganese salt of the factor with methanol. The final product had the properties of a nucleotide in that it contained nitrogen, phosphorus, and gave a positive Bial's test for pentose. A negative Feulgen test proved it was not desoxyribose. On the basis of hydrolytic studies, Stokstad felt that the active principle contained a purine and a pyrimidine nucleotide. Guanine but not adenine was present. This was in line with observations reported by Snell and Mitchell⁹ in which both purine and pyrimidine bases when added to the culture medium were found to give better growth responses in certain lactic acid bacteria.

Also in 1941, Mitchell, Snell, and Williams¹⁰ obtained a highly concentrated growth factor for *Strept. lactis R* from spinach. Because of the source of this material they suggested the name folic acid and defined it as a growth factor for this particular organism. The growth of *L. casei*, however, was also stimulated by folic acid. No phosphorus was present in this factor and one-half maximal growth was obtained at a level of 0.00012 γ per ml, while Stokstad's fraction required 0.014 γ per ml. Liver, yeast, and other substances were found to contain folic acid, and in preliminary studies on rats evidence suggesting intestinal synthesis was reported. Mitchell and Snell¹¹ described a microbiological assay method for folic acid using *Strept. lactis R* as the test organism. The amount of growth was measured by a thermoelectric turbidimeter or photoelectric colorimeter after 16 hours incubation. Standard curves were based on growth obtained between 20 and 200 micrograms of Wilson's liver extract fraction B (potency of one unit per microgram). The following year, 1942, Landy and Dickens¹² described a microbiological assay method for folic acid and other members of the vitamin B complex, using *L. casei* as the test organism. By the omission of certain single vitamins, no growth nor acid production was noted. The addition of increasing amounts of the missing vitamin up to a certain value gave proportionately increasing amounts of growth or acid production. The measure of growth could be made either by turbidimeter or by titration.

In 1943, Keresztesy, Rickes, and Stokes¹³ compared the amount of folic acid and norite eluate factor in various types of extracts and liver preparations and found that some of the materials were much more active for *Strept. lactis R* than for *L. casei*. This observation was in contrast to that found for the extract of spinach, which had the same degree of activity for both organisms. A new substance was then obtained from an unstated source which effectively replaced folic acid in the growth of *S. lactis*, but was inactive for *L. casei*. One gamma of the new material had the same potency for *S. lactis* as 56 γ of folic acid, but 1 γ was less active for *L. casei* than 0.0004 γ of folic acid. At approximately the same time

Stokstad¹⁴ isolated from liver and from yeast crystalline methyl esters which on hydrolysis yielded preparations of equal potency for *L. casesi*. The free acid of both fractions had the same absorption spectrum. When their microbiological potency was compared with the liver fraction B standard of Mitchell and Snell (see above), Stokstad's preparation from liver had a relative potency of 79,000 as determined with *L. casesi* and 78,000 with *S. lactis R*. The yeast preparation had a potency of 75,000 with *L. casesi* and 38,000 with *S. lactis R*. Thus the liver and yeast compounds differed from one another, and both seemed to be different from the growth factor isolated by Keresztesy et al. for *S. lactis R*. Stokstad believed that the liver factor was identical with the compound obtained by Pfiffner et al. (see below).

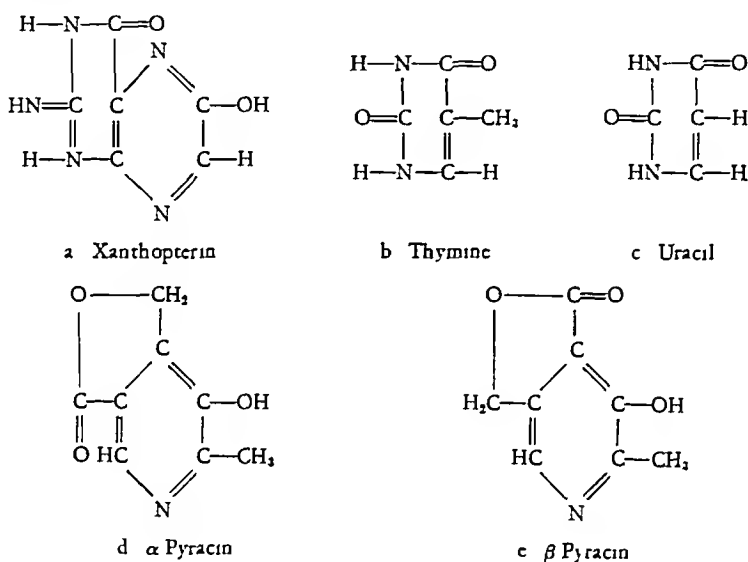


FIGURE 1

A series of four papers appeared in 1944 describing the isolation and properties of folic acid obtained from spinach. Mitchell, Snell, and Williams¹⁵ concentrated a product which was 137,000 times more active for *S. lactis R* than the standard liver product. The active principle was unstable to oxidation, reduction, acid, alkali, light, and heat and was highly reactive with many organic reagents. Attempts at crystallization were unsuccessful and all samples with potency exceeding 110,000 gave amorphous precipitates. In a special study of adsorption, Frieden, Mitchell, and Williams¹⁶ found that the elution of folic acid after adsorption on charcoal was much easier for crude preparations than for relatively pure solutions. This behavior was attributed to the presence of interfering substances which affected the manner of adsorption. In fact, adsorption isotherms indicated by a change in slope that the adsorption process was of a dual nature. This evidence, combined with certain chemical data, led Mitchell and Williams¹⁷ to the conclusion that most of the impurities present in their most concentrated preparations were of

a similar nature to the active compound. Analyses suggested an empirical formula of $C_{16}H_{16}O_8N_6$. No sugar or polyhydroxy group was indicated, but the presence of a xanthopterin-like structural unit was suggested (fig. 1a). Diffusion coefficient measurements compared to those of compounds of known molecular weight gave a value for folic acid of 400 ± 50 . In this paper the authors reported that a sample of folic acid with a potency of 75,000 had been tested by Norris for antianemic activity in trout. This test was suggested by an earlier report of Simmons and Norris¹⁸ that injections of 30–50 γ of xanthopterin from human urine brought about a cure of a nutritional anemia induced in Chinook salmon. Xanthopterin was just as effective in regenerating the blood as was liver extract. The folic acid, however, was only one-fifth as active as the xanthopterin since 10 γ per gram of fish was required for a good erythrocyte response. Mitchell¹⁹ obtained absorption spectra data to show that folic acid resembled xanthopterin. The light-absorbing structure was much more stable to light and acid treatment than the physiologically active compound. Difficulties in purification were thus attributed to inactive folic acid with physical properties only slightly changed.

Stokes, Keresztesy, and Foster²⁰ found that the *S. lactis* R factor which they had recently isolated¹³ would not support the growth of *L. casei* and other *Lactobacilli*. If 1 ml. of the supernatant from a 24 hour broth culture of *S. lactis* R containing one hundred times the amount necessary for optimum growth (0.3 γ unit versus 0.003 γ units) was added to a culture of *L. casei*, at least optimum growth was obtained. Thus SLR factor could be converted to folic acid by the streptococci and quickly enough to give identical growth curves when the two substances were compared. In fact these investigators found that all strains of bacteria capable of using the SLR factor could grow on folic acid to the same degree. This factor, however, unlike xanthopterin, did not give rise to folic acid when incubated with rat liver (see below). In their most recent paper Stokes and Larsen²¹ reported that resting cell suspensions of enterococci which required the SLR factor for growth, varied quantitatively in their ability to convert SLR factor to folic acid. *S. lactis* R cells grown in a medium containing the SLR factor were more active than those grown with folic acid or thymine. Within the range of 0.02 γ to 2.0 γ per ml. of suspension, the concentration of SLR factor in the growth medium did not affect the conversion significantly. Cells from cultures nearing the logarithmic phase formed more folic acid than younger cells. The transformation was rapid since maximum folic acid was obtained within two hours and the amount was proportional to both the cell density and SLR factor concentration. The presence of fermentable carbohydrates in the conversion mixture increased the yield of folic acid 4–20 fold, and the optimum pH was 8. After conversion, practically all of the folic acid was contained within the bacterial cell and it could be extracted by autoclaving or by digestion with clarase.

In addition to the above series of observations in which living cells were found capable of converting an inactive growth factor for a group of microorganisms into an active form, Mims, Totter, and Day²² produced from rat liver a relatively stable enzyme preparation which was capable of producing the SLR-stimulating factor from inactive material. The enzyme was extracted by treatment with ammonium

sulfate. The proof of such conversion was based on the demonstration that the amount of SLR factor increased following incubation of the material under test with the required amount of enzyme solution at 37° C. for four hours. The heat-coagulable protein was precipitated by immersing the tubes in a boiling water bath for three minutes. The supernatant liquid was then assayed for folic acid by the method of Mitchell and Snell (loc. cit.), using *S. lactis* R as the test organism. The magnitude of increase varied from 1-5 fold for potatoes to 100 fold for some samples of yeast. There seemed to exist, therefore, a potential folic acid factor readily convertible by the enzyme into an active form. In more recent work, Laskowski, Mims, and Day²³ have found that the enzyme has a very generalized distribution in the organs and tissues of various animals. For example, brain, pancreas, bone, intestinal mucosa, kidney, and spleen of the rat are rich sources of the enzyme, while muscle, heart, and liver have smaller amounts. Certain tissues of the dog, hog, cow, rabbit, and chicken also possess it. The unit of enzyme was established as the amount which produces 1 γ of the SLR factor (as folic acid of potency 40,000) per hour when incubated at 37° with 200 mgm. of yeast extract (Difco) in a total volume of 11 cc. and four hours total incubation. Partial purification of the enzyme was achieved by adsorption, precipitation, and salting out with Na₂SO₄. An average potency of 200 units per mg. of protein was obtained in a preparation from chicken pancreas. The optimal pH was between 7 and 8.

Barton-Wright, Emery, and Robinson²⁴ attempted to prepare folic acid from by-products of commercial liver extract manufacture. In addition to a fraction that could not be extracted from an aqueous solution by chloroform, other fractions soluble in chloroform and capable of stimulating growth of *L. casei* and *S. lactis* R were obtained. These fractions are believed to contain three or four growth factors, and the one insoluble in chloroform is thought to be folic acid.

From the foregoing discussion of the work with the folic acid group of substances required for the growth especially of *L. casei* and *S. lactis* R, it is apparent that it is impossible to understand clearly at the present time the interrelationships that exist. Optimal growth of both organisms may be obtained with the same factor. With other factors either *L. casei* or *S. lactis* R may show normal growth while the other grows to a much smaller degree. In such cases it is possible to convert by enzymatic or cellular means the inactive factor into an active form. As Luckey, Tepley, and Elvehjem²⁵ pointed out, part of the uncertainty associated with the work would disappear if more generally accepted methods of measuring units of activity or potency were used. In an effort to improve the reliability of microbiological assays for folic acid, Luckey, Briggs, and Elvehjem²⁶ determined the optimum amounts of biotin, nicotinic acid, pantothenic acid, pyridoxine, dipotassium phosphate, sodium acetate, tryptophan, glucose, and certain purines and pyrimidines for growth of *S. lactis* R on a casein hydrolysate-synthetic medium. A new medium was designed on the strength of their data. Tepley and Elvehjem²⁷ later modified this medium to allow more acid production for titrimetric determination of folic acid. Less scattering of assay values when different amounts of growth are obtained was achieved in this way. Another possible source of scatter was suggested by Sherwood and Singer²⁸ when they showed that the

grade of nonabsorbent cotton commonly used as plugs for bacteriological culture tubes contained appreciable amounts of folic acid. They used *L. casei* as the test organism and found that boiling the cotton for several hours in distilled water would remove most of the folic acid.

Before concluding this section of the review another series of observations should be emphasized and extended since they are of considerable theoretical significance and may be of practical importance for assay techniques. Snell and Mitchell,⁹ Stokstad,¹⁴ and Mitchell, Snell, and Williams¹⁸ have shown that certain pyrimidine and purine bases may be substituted for folic acid in the growth of both *S. lactis* R and *L. casei*. Of the pyrimidines used, thymine (fig. 1b) seems to be the critical component, with cytosine and uracil (fig. 1c) having little activity, whereas the purines (adenine, guanine, and xanthine) are more or less interchangeable. The replacement of folic acid with thymine was also reported by Mitchell and Williams¹⁷ and by Luckey, Briggs, and Elvehjem²⁶ in the growth of the test micro-organisms. Stokes¹ found that 5000 times as much thymine as folic acid was required for maximum growth of *S. lactis* and other enterococci and that a large number of pyrimidines, purines, nucleic acids, and similar compounds were inactive. Folic acid, moreover, could not be detected in cells which were grown on a medium containing thymine instead of folic acid. Stokes thus advanced the hypothesis that folic acid participates directly or indirectly as a coenzyme in the synthesis of thymine or some related compound in these micro-organisms. In testing more than 100 pyrimidines as growth factors for *L. casei*, Hitchings, Falco, and Sherwood³⁰ found 20 or more compounds of biological significance. Inhibition of growth was obtained with compounds having amino, thio, halogen, and various other substitutions in the pyrimidine molecule. These authors took exception to Stokes' hypothesis because bromouracil completely inhibited the growth of *L. casei* with thymine as the nutrient but had no effect when folic acid was present. Growth does not depend therefore on thymine synthesis as such. Moreover, nitrouracil prevented growth of *L. casei* with folic acid at concentrations which had little effect on growth with thymine. They suggest that the two nutrients may act as alternatives in a single system rather than as two components in the same system. Kreuger and Peterson³¹ emphasized the fact that thymine could completely replace vitamin B₉ (see below) in the nutrition of *S. faecalis* and could partially replace it in the nutrition of *L. casei*. In assaying material for vitamin B₉ by these test organisms as little as 1 gamma of thymine present free in the sample might erroneously influence the assay.

II VITAMIN M

As early as 1932, Wills and Bilimoria³² showed that monkeys on a diet comparable to that consumed by certain Indian natives, largely polished rice, white bread, and chapatti, developed anemia, leukopenia, and granulocytopenia. The bone marrow of these animals revealed a megaloblastic hyperplasia. Neither vitamin A nor C protected the animals, but the symptoms were relieved by yeast extract. Again in 1935, Wills and Stewart³³ produced a dietary anemia in the rhesus monkey in which the erythrocyte count dropped from five and a half million to less than two million and the leukocyte totals went from 16,000 to less than 5,000. Both the

anemia and the leukopenia were cured with marmite (a yeast extract preparation). The same year Day, Langston, and Shukers³⁴ fed young *Macaca mulatta* monkeys a diet believed to be adequate in protein, minerals, fatty acids, and vitamins A, B₁, C, and D but deficient in vitamin G (B₂) and possibly other less well known organic substances which may be essential. After a varying period on this diet the animals developed a fulminating, fatal blood disease characterized by leukopenia and anemia. Ulceration of the gums consistently accompanied the hypocythemia. Diarrhea was common. Brewer's yeast added to the diet supported good growth and prevented the deficiency syndrome. Day, Langston, and Shukers³⁵ in a brief paper listed the diet fed their monkeys as consisting of whole wheat, polished rice, purified casein, Osborne-Mendel salt mixture, cod-liver oil, and oranges. The anemia and leukopenia developed and terminated fatally in 26 to 93 days. A supplement of 10 grams of dried brewer's yeast daily supported normal growth and development in the animals for 400 days, while 2.5 grams daily was inadequate. Two grams of a liver-stomach preparation daily was satisfactory.

Wills, Clutterbuck, and Evans³⁶ compared the anemia induced in their monkeys by dietary means in from three months to one year to the tropical macrocytic anemia seen in Bombay. Marmite would protect against or cure this anemia as well as a fraction soluble in 80 per cent alcohol. Dried brewer's yeast was found not to be as effective as either a 0.1 per cent acetic acid extract or a 90 per cent alcohol extract. Heptoflavin was of no benefit, and wheat germ extracts were of value only when given in large doses. Day, Langston, and Darby³⁷ reported the failure of nicotinic acid in doses of 10 or 50 mg daily when used as supplements to protect monkeys from the fatal dietary cytopenia or to prolong life. This syndrome in the monkey was thus established as being different from blacktongue in dogs or pellagra in man. The factor preventing this condition was designated by these workers for the first time as vitamin M. A more extensive report appeared in the same year (1938) from the Arkansas group when Langston, Darby, Shukers, and Day³⁸ eliminated thiamin hydrochloride, riboflavin, and niacin, alone or in combination, as being responsible for the nutritional cytopenia of their monkeys. In addition to the 10 Gm of dried brewer's yeast daily which had previously been found to protect the animals from this blood disease, 2 Gm of a liver extract (Cohn fraction G) daily was shown to permit normal body development and maintain a normal blood picture over long periods.

As mentioned above,³⁴ the vitamin M deficient monkeys were commonly found to suffer from diarrhea. This aspect of the deficiency was studied in more detail by Janota and Dack.³⁹ They made blood counts and fecal bacteriological cultures before the animals were placed on the experimental diets. No dysentery organisms were found in these cultures. After one month on the deficient diet, one monkey gave positive stool cultures for bacillary dysentery and suffered from diarrhea, gingivitis, and leukopenia. By the third month seven had developed dysentery, while all of the controls remained healthy throughout this period. The following year Day, Langston, Darby, Wahlin, and Mims⁴⁰ isolated *Shigella paradysenteriae* from the stools of several vitamin M deficient monkeys (fed the Goldberger diet). The deficiency syndrome was similar to that found in the earlier work of this group,

and a crude liver extract given to an animal with profound anemia and leukopenia was found to elicit a dramatic reticulocyte response and ultimate recovery. The ash of liver extract had no protective value. Niacin and riboflavin seemed to have a mild erythropoietic effect.

Wilson, Doan, Saslaw, and Schwab⁴¹ placed monkeys on one of three diets deficient in the vitamin B complex. Leukopenia developed in from 30 to 105 days on these diets. A period of constant leukocyte counts was usually observed before a rather sudden onset of leukopenia. All cellular elements were involved in the cytopenia that terminated fatally. Only moderate degrees of anemia were observed in some monkeys. Intramuscular injections of folic acid concentrates corrected the leukopenia in those monkeys receiving other vitamin supplements. These observations were extended by Saslaw, Schwab, Woolpert, and Wilson⁴² to show that the monkeys while suffering from the nutritional granulopenic leukopenia exhibited a lowered resistance to spontaneous infections. The mortality rate in such infections was high. Saslaw, Wilson, Doan, and Schwab⁴³ then produced the vitamin M deficiency in monkeys on diets supplemented with riboflavin, thiamin hydrochloride, nicotinic acid amide, calcium pantothenate, pyridoxine hydrochloride, sodium para-amino-benzoate, choline chloride, pimelic acid, glutamine, and inositol. Only half of the animals developed significant degrees of anemia, but all developed leukopenia in from 4 to 15 weeks. Three monkeys received limited supplements of a yeast residue containing folic acid during a brief experimental period. A marked leukopoiesis and remission of clinical symptoms followed. The animals supplemented with the above vitamins plus 2 cc. of a crude liver extract did not develop the cytopenia.

Waisman, Rasmussen, Elvehjem, and Clark⁴⁴ made a study of the nutritional requirements of the rhesus monkey employing a diet composed of sucrose, casein, salts, corn oil, and supplements of 8 members of the vitamin B complex and vitamin C. None of the animals could survive on this diet, and all showed weight loss, anorexia, moderate degrees of anemia, leukopenia, and extensive and varied lesions attributed to secondary infections with various organisms, especially those of bacillary dysentery. A biotin concentrate did not alter the course of the deficiency. Liver or liver extract prevented the deficiency. Waisman and Elvehjem⁴⁵ then found that a norite eluate fraction of liver containing folic acid readily cured the vitamin M syndrome. However, they attributed part of the response to the role that folic acid might play in increasing the synthesis of biotin by intestinal bacteria. This conclusion was based on the observation that the loss of hair in the deficient monkey was not corrected by the addition of 1 per cent solubilized liver to the diet. The leukopenia was cured, but since the solubilized liver was low in biotin the return of fur in an animal was obtained only when folic acid was also given to the monkeys.

The anemia and leukopenia of vitamin M deficient monkeys was alleviated by daily supplements of 0.5-10 mgm. of synthetic xanthopterin according to Totten, Shukers, Kolson, Mims, and Day.⁴⁶⁻⁴⁷ A subnormal reticulocyte response of 15-45 per cent (normal, 0.2-0.4 per cent) resulted in 3 to 6 days and was maintained for 2 to 5 days. The blood cells reached normal values in 3 to 13 days and remained

normal for varying periods. It was only in the animal that received a heated liver powder in addition to the xanthopterin that the hemocytopoietic function was restored to normal for an extended period. After 71 days the blood was normal, but when the xanthopterin was withheld cytopenia promptly returned. The resumption of xanthopterin elicited a second response similar to the first. Xanthopterin alone, however, did not seem to fulfill completely the role of vitamin M, but it did delay the onset of the symptoms. Totter, Mims, and Day⁴⁸ compared the folic acid content of several substances with the vitamin M activity in monkeys. The amount of folic acid present as measured by growth of *S. lactis* R could account for only a small proportion of the vitamin M activity. There was much better agreement, however, between the vitamin M activity of a substance and its folic acid content if the material was first incubated with fresh rat liver before being assayed with *S. lactis* R. This was especially true when yeast was the substrate. The livers of two vitamin M deficient monkeys were low in preformed folic acid. Extra folic acid was produced from xanthopterin by one liver, but the other liver required yeast in addition to the xanthopterin. This latter requirement was found to prevail with chicken liver.

Day, Mims, Totter, Stokstad, Hutchings, and Sloan⁴⁹ treated three monkeys made cytopenic by a deficient diet with 4 to 4.5 mg. of a highly purified preparation of *L. casei* factor per day for several days. There was a prompt and complete recovery. Total leukocyte and granulocyte counts had increased from normal to 7.8-10.9 per cent. Erythrocyte counts increased more slowly to normal. The infections commonly associated with this deficiency were found in one of the monkeys, and these healed following the treatment. This *L. casei* factor was only slightly active for *S. lactis* R, but treatment with the enzyme described by Mims et al.²² increased the activity for this micro-organism. Day, Mims, and Totter⁵⁰ made further tests with the crystalline *L. casei* factor in their vitamin M deficient monkeys, using 3 mg. intramuscular injections over several days in each case. Reticulocytes rose as high as 47 per cent within 4 to 7 days in these animals. Erythrocytes, hemoglobin, and leukocytes returned to normal and the dose maintained the remission for 10 to 30 days. These blood changes were accompanied by marked clinical improvement. These authors likened this factor to vitamin B₆ conjugate (see below) in its microbiological activity.

III. VITAMIN B₆

Studies in nutrition of the chick have yielded valuable information in many aspects of vitamin research. It is not surprising to find therefore that some of the early investigations with what is now commonly referred to as folic acid made use of this experimental animal. Stokstad and Manning⁵¹ reported that chicks on a diet adequate in all the essential elements known at the time (1938) required an additional factor for growth which could be found in yeast, middlings, alfalfa, and wheat bran. Certain stability characteristics were described. The following year, Hogan and Parrott⁵² published in abstract form and later in detail⁵³ the results of experiments proving the existence of a new member of the vitamin B complex required by the chick. In the absence of this factor the animals grew slowly and de-

veloped a macrocytic hyperchromic anemia. The erythrocytes were less fragile but the coagulation time was normal. The anemia was not due to anorexia and could not be prevented by any of the known vitamins. A water soluble extract of liver was required. Because of its role in the chick, the protective substance was designated as vitamin B₆. Two other papers appeared in 1940 in which factors from yeast were found to be required for growth in chickens. Stokstad, Manning, and Rogers⁵⁴ proved that a substance other than pyridoxine was required, and Schumaker, Heuser, and Norris⁵⁵ felt that their factors R and S from yeast were probably related to those previously described by Stokstad. In neither publication was any mention made of the possibility of anemia developing, but it was stated by Schumaker et al. that no macroscopic lesions appeared.

Hutchings, Bohoyos, Hegsted, Elvehjem, and Peterson⁵⁶ in 1941 prepared from liver a concentrate 200 times as active as the original material in promoting the growth of *L. casei*. They then showed that the amount of this material required for the growth of the chick was decreased in proportion to its increased activity for *L. casei*. They concluded that the two active principles might not be identical but that the data suggested that they were. The following year, Mills, Briggs, Elvehjem, and Hart⁵⁷ used a basal medium which was fed day old white Leghorn chicks. These animals grew poorly and at the end of four weeks had an average hemoglobin value of 6.13 Gm. per 100 ml. blood, while chicks receiving supplements of solubilized liver extract or norite eluate factor from solubilized liver grew much better and had hemoglobin values of 8.40 and 8.09 Gm. per 100 ml. blood respectively. These experiments gave further support to the identity of vitamin B and the *L. casei* factor.

O Dell and Hogan⁵⁸ undertook an investigation to improve the diet used for producing anemic chicks which might then be used in vitamin B₆ assays. A diet was found that gave an incidence of 53 per cent anemia in the animals. They suggested that higher levels of pyridoxine in the diet tended to lower the incidence of anemia because it aided in the bacterial synthesis of the antianemic principle. In support of this hypothesis was the larger percentage number of anemic animals obtained when sulfaguanidine was added to the diet. It was of interest that the eggs of hens consuming a diet rich in green foods yielded fewer anemic chicks than when the eggs came from hens fed a dry diet. Apparently vitamin B₆ was stored in the egg when the diet was rich in this factor. Chemical studies of the vitamin showed it to be an acid capable of forming salts with heavy metals. It could be adsorbed from acidic solution by a variety of adsorbents and was eluted with ammonia. These properties are in close agreement with those found for the various growth factors (compare above).

A brief account was published in 1943 by Pfiffner, Binkley, Bloom, Brown, Bird, Emmett, Hogan, and O Dell⁵⁹ in which some of the physical characteristics of a crystalline compound isolated from liver were described. This acidic substance when fed to day old chicks at a level of 2.57 per gram of deficient ration insured normal growth and the animals showed no anemia at the end of four weeks. It was highly active as a growth factor for *L. casei* since a concentration of 0.0005 per ml. of culture medium gave half-maximum growth of the organism. They thought

that this crystalline material was probably the same as Peterson's eluate factor and Williams' folic acid. The term vitamin B₆ was retained in order to designate the pure crystalline compound they had isolated. Campbell, Brown, and Emmett⁶⁰ in a more quantitative experiment showed that vitamin B₆ would prevent the nutritional macrocytic hyperchromic anemia in chicks at levels of 40 γ per 100 Gm. of deficient ration. Growth required 100 γ and a normal leukocyte level required 400 γ per 100 Gm. of ration.

The ultraviolet absorption spectrum of vitamin B₆ was measured by Bloom, Vandenberg, Binkley, O'Dell, and Pfiffner⁶¹ at pH values between 1.0 and 11.0 and compared with the absorption of xanthopterin at essentially the same hydrogen ion concentrations. Very striking dissimilarities were found although in general the curves were alike. Similarities were also found in curves for flavins, alloxazines and pterins.

Binkley, Bird, Bloom, Brown, Calkins, Campbell, Emmett, and Pfiffner⁶² found that yeast extracts were highly potent in vitamin B₆ activity as measured in the anemic chick, but only 2-5 per cent of the chick antianemic activity showed in the microbiological growth effect on either *L. casei* or *S. lactis*. When this yeast concentrate was subjected to enzymatic digestion it became microbiologically active. The chemical procedure for crystallizing vitamin B₆ was applied to the yeast digest. A crystalline compound was obtained that had the same activity for *L. casei* and *S. lactis* as the compound from liver. It also had a comparable effect on growth and on the blood picture in the chick. The crystalline compounds from liver and from digested yeast had identical crystallography, ultraviolet absorption spectra, and elemental chemical analyses. These observations suggested that the vitamin B₆ activity in yeast extract was due almost entirely to a relatively simple nonprotein conjugate of the vitamin. Work with this crystalline vitamin B₆ conjugate from yeast was continued by Pfiffner, Calkins, O'Dell, Bloom, Brown, Campbell, and Bird.⁶³ The ultraviolet absorption spectrum when compared with that of crystalline vitamin B₆ was found to differ only in values. This indicated that the two compounds had the same chromophoric groups but that the molecular size of the conjugate was 2.8 times that of vitamin B₆. Microbiological assay data showed that 1 γ of conjugate was equivalent to 0.003-0.006 γ of vitamin B₆ as measured by *L. casei* and to only 0.002 γ using *S. lactis*. An enzyme called vitamin B₆ conjugase isolated from hog kidney released an amount of vitamin B₆ from the crystalline vitamin B₆ conjugate approximating the amount present in conjugated form as calculated from ultraviolet absorption data. The compounds from both liver and yeast gave comparable antianemic activity in the chick. From these data the authors believed their yeast compound to be different from those of Stokstad.⁵¹⁻⁵⁷ Bird, Binkley, Bloom, Emmett, and Pfiffner⁶⁴ showed that the hog kidney extract, mentioned above, when added to the yeast vitamin B₆ conjugate released the maximum amount of vitamin B₆ within approximately four hours as measured by the growth response of *S. lactis*. More recently Bird, Bressler, Brown, Campbell, and Emmett⁶⁵ described the enzymatic liberation of vitamin B₆ from the bound form as measured with growth of *L. casei*. The enzyme was obtained from desiccated hog kidney and from extracts of whole almond or almond meal. The microbiological

assays of various materials, following the enzyme treatment, were for the most part in agreement with chick antianemic assays. Campbell, McCabe, Brown, and Emmett⁶⁶ found that 20-40 γ of crystalline vitamin B₆ per 100 Gm of ration would prevent the anemia, leukopenia, and thrombocytopenia in chicks during the first four weeks of life. Even 400 γ per 100 Gm of ration did not overstimulate the production of cellular elements.

In 1944, Hutchings, Stokstad, Bohonos, and Slobodkin⁶⁷ isolated in crystalline form a new compound from an unspecified source which was active for both *L. casei* and *S. lactis* R and was also active in the nutrition of the chick. It was 85-90 per cent as active as the compound from liver¹⁴ when assayed with *L. casei*, but it was only 6 per cent as active when tested by *S. lactis* R assay. The new compound was required in amounts of 0.000061 γ per ml for *L. casei* and 0.0042 γ per ml for *S. lactis* R. Absorption spectrum data made for this compound and the two previously isolated¹⁴ appeared to be different from Mitchell and Williams^{17, 19} folic acid.

Two dietary factors necessary for the chick were found in liver by Briggs, Luckey, Elvehjem, and Hart.⁶⁸ On the basis of microbiological assays these factors were distinct from folic acid. One factor was essential for proper feather development, and the other was required for growth. They were designated vitamins B and B₁₁. Hill, Norris, and Heuser⁶⁹ confirmed the earlier evidence⁶⁸ for the existence of two chick growth factors, R and S, from yeast distinct from folic acid or vitamin B₆. If folic acid and vitamin B₆ are the same, a new chick antianemic factor distinct from factors R and S was thought to have been revealed. This antianemic factor might have been vitamin B₆ in the event that crystalline preparations of it were contaminated with highly potent growth and antianemic factors or stimulated the bacterial synthesis of these factors in the intestinal tract. No evidence was obtained that folic acid was required by the chick, since growth failure and mortality occurred in the absence of the antianemic factor and factor R or S. Campbell, Brown, and Emmett⁷⁰ tested the hypothesis that vitamin B₆ might alter bacterial synthesis of other vitamins in the intestine. Growth, feathering, and the blood picture of chicks were observed following oral and subcutaneous administration of the vitamin. The results for both groups were the same. Briggs, Luckey, Elvehjem, and Hart⁷¹ reported that vitamins B₁₀ and B₁₁ were distinct from folic acid when measured by *S. lactis* R and *L. casei*. Chicks fed a basal diet developed macrocytic hyperchromic anemia which was partially cured by supplements with vitamin B activity. They felt that another factor was concerned with hemoglobin formation since fractions low in vitamin B₆ activity raised the hemoglobin value considerably. Fractions rich in vitamin B₁₀ or B₁₁ did not completely prevent the anemia.

At the end of the summer of 1945 a large group of investigators reported the synthesis of a compound which was active for *L. casei*, *S. lactis* R, and was effective in promoting growth and hemoglobin formation in the chick. The synthetic compound was believed to be identical with the crystalline *L. casei* factor previously isolated from liver. This conclusion was based on several observations. Ultraviolet absorption spectra of the two compounds were identical. The refractive indices of light vibrating parallel to the length of the crystal and also to the width

of the crystals were identical for both compounds. The compounds were equally active when assayed with *L. casei* or *S. lactis* R. No data were given concerning the chemistry of this compound or its possible relationship to xanthopterin. As yet none of this information has been published.

It is clear that the work with the chick leaves several problems yet to be resolved. There is the possibility that vitamins B₁₀ and B₁₁ and factors R and S are related. They may represent conjugated forms of vitamin B_c which fail to reveal their potency for the chick when assayed by micro-organisms. Until this contingency is clearly eliminated, doubt must remain as to their distinction from folic acid. There is, on the other hand, newer evidence that indicates *L. casei* factor alone is inadequate for a normal blood picture in chickens. Scott, Norris, Heuser, Bruce, Coover, Bellamy, and Gunsalus⁷³ reported in a note that the lactone of 2-methyl-3-hydroxy-4-hydroxymethyl-5-carboxypyridine (fig. 1d) promotes growth and prevents anemia in chicks. The use of this synthetic compound was suggested by the observation that pyridoxine previously treated with hydrogen peroxide promoted the growth of *L. casei*. Scott, Norris, Heuser, and Bruce⁷⁴ showed that the above lactone, now designated as α pyracin, or the isomeric 4-carboxy lactone, β pyracin (fig. 1e), was required for the complete prevention of the macrocytic hyperchromic anemia that develops in chicks fed a purified diet. Of the two compounds, β pyracin was more active in promoting growth but was only slightly more active in preventing anemia. Smaller quantities of the compound prevented anemia than were required to promote growth. Hematological studies revealed that when *L. casei* factor alone was added to the diet a normocytic, hypochromic anemia developed, whereas the addition of β pyracin alone led to the development of a macrocytic normochromic type. More recently, Scott, Norris, and Heuser⁷⁵ produced a severe hemorrhagic anemia in hens by withdrawing by cardiac puncture a volume of blood estimated as one-third the total volume. Hemoglobin regeneration was then followed daily, and it was found that injections of β pyracin and *L. casei* factor, alone or in combination, hastened the recovery process. The administration of these factors together gave higher hemoglobin values which were maintained longer than in the hens receiving only a single factor.

Briggs and Lillie⁷⁶ observed that day old chicks fed a highly purified diet deficient in folic acid for four weeks developed characteristic deficiency symptoms such as poor growth, retarded and rough feathering, anemia, perosis, and high mortality. The survivors were then given a normal broiler ration which supported growth and feathering. After 3 to 6 weeks on this ration, however, many of the chicks developed wing and body feathers which contained large white areas and often abnormal black areas. When Wilson's Liver Fraction L or synthetic *L. casei* factor (Lederle) was included in the diet during the first four weeks, the chicks developed normally in all respects.

The various factors which have been isolated in work on micro-organisms, monkeys, and chicks are summarized in table 1. Our knowledge of some of these factors is obviously fragmentary, and until the chemistry of synthetic *L. casei* factor becomes available it will be difficult to understand the possible interrelationships which seem to exist in these factors. Since there have been so many different sub-

stances reported in the literature, it is felt that such a tabulation might serve as a guide to the reader

TABLE I

Name	Source	Biological Activity	Chemical Nature	Reference
Norite eluate factor	Liver and yeast	Growth factor for <i>L. casesi</i> and <i>S. lactis</i>	Basic, related to purines	6
Norite eluate factor	Solubilized liver	Same as above	Acidic not a nucleotide	7
Norite eluate factor	Solubilized liver	Same as above	Purine and pyrimidine present	8
Folic acid	Spinach	Same as above	Not a nucleotide	10
<i>S. lactis</i> R factor	Unstated	Active for <i>S. lactis</i> Inactive for <i>L. casesi</i>		13
Crystalline <i>L. casesi</i> factor	Liver and yeast	Liver factor active for both organisms, yeast factor half active for <i>S. lactis</i>	Methyl esters of active principle	14
Folic acid	Spinach	Same as folic acid above	Xanthopterin like structure	15, 16 17, 19
Marmite	Yeast	Cures dietary anemia in monkeys		32
Vitamin M	Liver and yeast	Cures nutritional cytopenia in monkeys		37, 38
Xanthopterin	Synthetic	Partially cures cytopenia in monkeys Cures trout anemia		46, 47 18
Vitamin B ₁₂	Liver	Cures chick anemia		53
Factors R and S	Yeast	Required in chick nutrition		55
Crystalline Vitamin B ₁₂	Liver	Active for chick and <i>L. casesi</i> <i>S. lactis</i>	Acidic, similar to flavins aloxazines and pterins	59, 61
Crystalline Vitamin B ₁₂	Yeast Digest	Same as above	Same as above	60
Crystalline Vitamin B ₁₂ conjugate	Yeast	Active in chick 2-5% activity for <i>L. casesi</i> and <i>S. lactis</i>	Same as above, molecule 2.8 larger	60, 63
Crystalline <i>L. casesi</i> factor	Unspecified	Active for <i>L. casesi</i> Inactive for <i>S. lactis</i>	Absorption spectrum unlike folic acid	60
Vitamins B ₁₀ and B ₁₁	Liver	Active for chick Inactive for bacteria		60, 71
Thymine	Synthetic	Active for <i>S. lactis</i>	Pyrimidine	69
Crystalline <i>L. casesi</i> factor	Synthetic	Active for chick rat monkey bacteria		70
α pyracin and β pyracin	Synthetic	Hemoglobin synthesis in the chick	Lactone of pyridine	73, 74 75

IV THE ROLE OF FOLIC ACID IN NUTRITION OF THE RAT

This topic has recently been reviewed by Daft and Sebrell⁷⁷ in connection with a more general discussion of the relationship between sulfonamides and vitamin

deficiencies. For a complete presentation of the topic, however, we feel justified in duplicating some of their effort.

The definite beginning in work of this type is sometimes difficult to ascertain. Nevertheless, in 1937 Gyorgy, Goldblatt, Miller, and Fulton⁷⁸ found that rats on a diet deficient in vitamin B₆ developed agranulocytosis, thrombocytopenia, and anemia. This pancytopenia could not be corrected by the addition of pyridoxine but required Peter's eluate. Also in 1937, Tschesche and Wolf⁷⁹ reported that xanthopterin, as well as other pterins, would give a reticulocyte response and rise in red cell counts in rats with a goat's milk anemia. A liver preparation and also a mixture of tryptophan and histidine were also effective.

Unna, Richards, and Sampson⁸⁰ found that rats on a diet deficient in pantothenic acid, biotin, and folic acid developed achromotrichia, retardation in growth, inflammation of the nose, and adrenal hemorrhages. The condition was prevented with pantothenic acid, but liver and rice exerted a better growth-promoting effect. Martin,⁸¹ on the other hand, added 1 to 2 per cent sulfaguanidine to a highly purified diet containing adequate amounts of calcium pantothenate. There was marked graying at 5 months in these animals with or without the addition of biotin. Both yeast and liver caused a sharp increase in growth rate and the graying was completely cured in two months on these supplements. When 3 mg of a folic acid concentrate per day was substituted for the yeast or liver, growth gains were normal. Graying was cured completely in three rats and was only partial in the other two.

Nielson and Elvehjem⁷² studied the growth-promoting effect of folic acid and biotin in weanling rats fed 1 per cent succinylsulfathiazole. After 4 to 5 weeks on the diet, at which time a weight plateau or loss began, the vitamins were tested. Biotin alone gave very low growth responses, but when it was added to the concentrate of folic acid the definite growth responses obtained with the folic acid alone were improved. Welch and Wright⁸³ and Wright and Welch⁸⁴ added succinylsulfathiazole to highly purified diets containing all the dietary factors known for the rat. In addition to growth inhibition they observed an increase in the prothrombin time. Both deficiency symptoms were overcome by the addition of folic acid and crystalline biotin. These workers suggested that these factors promoted bacterial synthesis of other essential dietary factors in the intestine. In fact, the appearance of achromotrichia and porphyrin-caked whiskers was accompanied by a pronounced reduction in the pantothenic acid content of the liver. These signs disappeared when folic acid and biotin were added to the diet. Their role is probably secondary to that of pantothenic acid in such cases.

The development of agranulocytosis, leukopenia, and a hypocellularity of bone marrow was reported by Spicer, Daft, Sebrell, and Ashburn⁸⁵ for rats fed either sulfaguanidine or sulfasuxidine at 1 per cent level in purified diets. Whole dried liver extracts would either prevent or cure these symptoms. Ashburn, Daft, Endicott, and Sebrell⁸⁶ observed that these deficient animals developed hyaline sclerosis and calcification of blood vessels, necrosis of voluntary muscles, and granulocytic aplasia of the bone marrow. Certain liver fractions which were known to contain the *L. casei* factor when administered orally to deficient rats were found by Kornberg, Daft, and Sebrell⁸⁷ to correct in four days the granulocytopenia and to restore

the red blood cells to normal in ten days. These recoveries were obtained during the continued ingestion by these animals of the sulfonamide-containing diet. Daft and Sebrell⁸⁸ treated rats on the sulfonamide diet with 20-40 γ of xanthopterin per day for four days without a response when the leukocytes had reached a level of 400 or less per cu mm and the granulocytes were 200 or less. Crystalline folic acid given orally to similar animals in doses of 20 γ for four days gave an average rise of from 2700 white cells per cu mm to 14,400 cells per cu mm. The granulocytes changed at the same time from 1 to 39 per cent. Erythrocytes increased in ten days from 5.1 million to 6.9 million, and the hemoglobin rose from 9.7 to 12.8 Gm.

Axelrod, Gross, Bosse, and Swingle⁸⁹ also found leukopenia and sometimes anemia in rats fed purified diets containing 1 per cent sulfaguanidine. The test substances were given orally for seven days, and both norite eluate and whole liver were effective in correcting the deficiency in leukocytes and in hemoglobin. Ransone and Elvehjem⁹⁰ tested the effectiveness of a folic acid concentrate in counteracting the decreased growth and leukopenia in rats fed sulfasuxidine in the diet and obtained approximately the same results as they did with a liver extract when each was fed at levels equal in *S. lactis* R activity. Biotin increased the growth rate when it was fed in conjunction with folic acid even when the latter was fed in insufficient quantities. Xanthopterin fed in conjunction with biotin did not increase the growth rate of the rats on the diet containing sulfasuxidine. In contrast to these observations, Totter and Day⁹¹ reported an immediate weight gain in rats on a similar diet but also containing biotin when 20 γ of xanthopterin were added. They noted a pronounced leukocyte response in which the average white cell count increased from 3420 per cu mm to 9400 per cu mm. They stated, however, that a normal distribution of white blood cells following xanthopterin therapy did not occur and that normal growth was not fully restored. It was suggested that this might be due to the injurious action of the drug as well as to the inadequacy of the xanthopterin. On the strength of the work of Totter and Day, Mitchell⁹ pointed out that analyses of some of his folic acid concentrates showed that xanthopterin was not infrequently present as an impurity. He recommended caution in attributing the response to folic acid alone in rats on a sulfonamide diet receiving folic acid concentrates.

Mallory, Mims, Totter, and Day⁹² fed yeast extracts or yeast extract concentrates to rats made leukopenic by adding succinylsulfathiazole to the diet. These substances were low in preformed *S. lactis* R stimulating factor, but the animals grew better and maintained higher total white blood cells and granulocytes than those receiving liver extract containing 1.9-15 times more preformed SLR factor. Thus there was no correlation between the effectiveness of these substances in sulfonamide-fed rats and their content of preformed SLR factor. But when the activity in rats was expressed as the amount of potential SLR factor present in the supplement, close agreement was obtained. These findings suggested that the factor antagonistic to the sulfonamide effect in rats was the same as or similar to vitamin M, since both could be converted into a growth stimulator for *S. lactis* R.

In rats fed a highly purified diet adequate in the known required members of the vitamin B complex, Wright and Welch⁹⁴ obtained a marked reduction in the

amount of folic acid and biotin stored in the liver compared with the amounts found in livers of rats fed a stock ration. The amount of these factors in liver was further reduced when succinylsulfathiazole was included in the diet. In addition there was also a reduction in the amount of pantothenic acid in the liver of these animals. The administration of crystalline biotin and a concentrate of folic acid not only caused a prompt restoration of growth but there was a recovery from the signs of pantothenic acid deficiency and a return to normal levels of the liver content of this vitamin. Welch and Wright⁹⁶ observed that rats on a diet of powdered whole milk supplemented with minerals and the known vitamins showed no evidence of nutritional deficiency when succinylsulfathiazole was fed in amounts as large as 10 per cent. This was in sharp contrast with the usual symptoms produced at 1-2 per cent levels. The assays for folic acid in the livers of animals fed a milk diet without sulfonamide were larger than those for the livers of rats fed a highly purified diet which contained the same original amount of microbiologically active folic acid. These observations were considerably clarified in a report by Wright, Skeggs, Welch, Sprague, and Mattis⁹⁶ in which the typical syndrome of the sulfa-induced deficiency was obtained in rats on the powdered milk diet with 10-20 per cent succinylsulfathiazole. Large amounts of folic acid were found in the feces of animals fed exclusively on powdered milk, but this elimination of folic acid was reduced by feeding the sulfonamide. Even so, a folic acid deficiency existed in rats showing considerable fecal elimination of the vitamin. By means of digestion technics it was shown that the milk contained significant amounts of potential folic acid which was unavailable to the micro-organisms used in assaying the diet. The rats were apparently capable of utilizing the potential folic acid in milk and therefore were more resistant to the sulfonamide-induced deficiency on the milk diet. Wright, Skeggs, and Welch⁹⁷ then demonstrated that folic acid may undergo conversion in the liver of rats into materials having little or no activity as growth factors for lactic acid organisms. Evidence was obtained by showing that the folic acid content of rat liver was increased by NaCl, xanthopterin, greater dispersion of tissue, and at pH 7-8. Cyanide inhibited the yield.

Endicott, Kornberg, and Daft⁹⁸ reinvestigated the lesions found in rats on purified diets containing one of several members of the sulfa drugs. A depletion of mature granulocytes in bone marrow with or without an increase in nucleated erythrocytes occurred. A few animals had normal marrow, and a few with normal peripheral blood had depleted marrow. Aplastic marrow was not observed in any of these rats as it was previously⁸⁶ but there was no explanation for this difference. Hyperplasia was regularly found in rats recovering from the granulocytopenia following the administration of liver concentrates. Gross, Axelrod, and Bosse⁹⁹ also found severe pathological changes in rats under similar experimental conditions which became maximal after two months. They had 90 per cent fatalities, which were reduced to 14 per cent by the administration of folic acid concentrate plus biotin.

Kornberg, Daft, and Sebrell¹⁰⁰ observed granulocytopenia (designating less than 500 granulocytes per cu mm blood) in 6 of 185 weanling rats fed a purified diet without sulfonamide. All of these deficient animals showed weight loss or poor growth prior to treatment with crystalline *L. casei* factor, and one animal died

The administration of the vitamin increased the polymorphonuclear cells to more than 5000 per cu mm of blood and restored growth. One or more relapses occurred, but the crystalline *L. casesi* factor corrected the granulocytopenia in relapse. These important observations eliminated the possible toxic influence of the sulfonamides in inducing the folic acid deficiency in rats. Kornberg, Tabor, and Sebrell¹¹ produced severe anemia in rats fed a purified diet containing sulfasuxidine. Anemia was noted in only a few rats not bled but fed sulfonamide, and none showed such severe anemia when fed a purified diet without sulfonamide even after long periods of bleeding. Crystalline *L. casesi* factor was fed as a supplement to one group of rats on the purified diet plus sulfasuxidine. The hematocrit average was 44 per cent six days following the eleventh bleeding made during a 24 day period. Unsupplemented litter mates under identical conditions had a hematocrit average of 19 per cent. The average hemoglobin was 14.8 Gm. in the supplemented group and 6.6 Gm. in the other. Of 31 rats made anemic by this procedure, 13 were treated with the *L. casesi* factor. Nine showed an average hematocrit rise of from 15 per cent prior to treatment to 28 per cent four days after treatment. The remaining untreated rats died in an average of seven days following the last bleeding.

Higgins¹⁰² fed young male rats a purified ration supplemented with the known vitamins. Sixteen were given 80 γ of vitamin B₁₂ concentrate per day for 14 days while receiving 50 mg. of promin daily. Twenty-nine were given the same amount of drug and no vitamin, while 15 were given the drug and were then treated. In all cases the administration of the vitamin had a marked antianemic effect with an average increase in hemoglobin from 11.7 Gm. per 100 cc. to 14.2 Gm. per 100 cc. blood. The erythrocyte volume and total red cell count showed slight increases.

A nutritional anemia in rats has recently been reported to occur after protracted periods on a purified diet. A morphological difference distinguishes this anemia from that due to a pantothenic acid deficiency and occurs in animals receiving this vitamin. Carter, MacFarlane, O'Brien, Robb-Smith, and Amos¹⁰³ suggest that it may be due to folic acid deficiency.

V FOLIC ACID IN THE NUTRITION OF OTHER ANIMALS

As early as 1939, Wintrobe, Samter, and Lisco¹⁰⁴ showed that weanling pigs fed a purified diet containing yeast grew satisfactorily. If thiamine, riboflavin, and nicotinic acid were substituted for the yeast, a severe anemia developed. When yeast was returned to the diet, partial or complete recovery followed. Folic acid may have been responsible at least in part for the therapeutic effect of yeast in these experiments, but the author's work antedated the discovery of this vitamin.

In 1942, Woolley¹⁰⁵ fed guinea pigs a ration composed of casein, sucrose, inorganic salts, corn oil, and vitamins A, C, D, E, K, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, choline, and inositol. The animals failed to grow and soon died unless two new factors were added to the diet. One was soluble in 50 per cent alcohol (GPF-1) and one was insoluble in the alcohol (GPF-2). The former was successfully concentrated so that 5 mg. per day produced good growth. Woolley and Sprince^{106, 107} later succeeded in proving that crystalline vitamin B₁₂ was one of the unknown factors required by the guinea pig but another as yet unidentified factor remained.

Nielson and Black,¹⁰⁸ using a purified basal ration, noticed that the albino mouse failed to grow after four weeks and developed a rough coat and curvature of the spine. The addition of folic acid to the diet supported better growth than the basal ration, but when biotin was also included normal growth and appearance resulted. In a study of spontaneous breast cancers in mice, Leuchtenberger, Leuchtenberger, Laszlo, and Lewisohn¹⁰⁹ observed spontaneous regressions in the cancers of 38 out of 89 animals (43 per cent). The treatment consisted of daily parenteral injections of 5 γ of *L. casei* factor. The incidence of new tumor development was decreased among the treated mice when compared with the controls.

A series of studies¹¹⁰⁻¹¹² was made in which the B vitamin content of various tissues was assayed during the development of rats and of chicks. The problems associated with autolyzing such tissues for assay were described and techniques were recommended. The fact that folic acid was destroyed during autolysis in acid or in alkali was emphasized. It was further demonstrated that bound folic acid in some tissues rendered the evaluation difficult.

An interesting relationship between stilbestrol and *L. casei* factor was revealed by Hertz and Sebrell.¹¹⁴ Normally, stilbestrol induces a marked proliferation of the tissues of the genital tract in the chick. This is reflected in an increase in weight of the genital organs and may be employed as an index of hormone activity. Chicks were maintained from hatching on a purified diet with supplements known to produce a vitamin B₆ deficiency, 0.5 mgm of stilbestrol in 0.1 cc of corn oil was given subcutaneously on each of six days. The weight of the oviducts of these chicks was consistently less than the weight of those in chicks on the same diet receiving supplements of *L. casei* factor. The weight difference was obtained whether the *L. casei* factor was administered from hatching or only as a curative measure during the last ten days preceding autopsy. Since the response to stilbestrol was unimpaired in pantothenic acid deficient chicks, debility limitations of growth were not sufficient to affect a reduction in oviduct response observed in the folic acid deficient chicks. These data indicated that an adequate intake of *L. casei* factor was essential for normal metabolism of stilbestrol in these animals.

Krehl and Elvehjem¹¹⁵ placed young dogs on a synthetic ration low in nicotinic acid. A severe deficiency resulted. There was a poor response to added niacin which was soon followed by a relapse and death despite the administration of this vitamin. When the basal ration was supplemented with a folic acid concentrate, a nicotinic acid deficiency was obtained which responded adequately and consistently to nicotinic acid.

In the field of invertebrate nutrition, Tatum¹¹⁶ showed that the larvae of *Drosophila melanogaster* grown under sterile conditions required a water-soluble yeast extract and an insoluble residue of yeast autolysate for normal growth. The basal agar medium contained amino acids, carbohydrate, salts, and thiamine, riboflavin, pyridoxine, nicotinic acid, and calcium pantothenate. More recently, Golberg, De Meillon, and Lavoipierre¹¹⁷ in studies of growth factors required for the larvae of *Aedes aegypti* L. found that folic acid was necessary for pupation. Its effect could not be replaced by xanthopterin or thymine. Folic acid also exerted an important effect on growth and survival rates, body pigmentation, and size of the larvae. Transference of the larvae from a folic acid-free medium to one containing the

vitamin, and vice versa, revealed that the most vital effect seemed to occur during the third stage of larval life. Kidder¹¹⁸ obtained quantitative data to prove that the increase in population of his ciliate, *Tetrahymena geleii* W, grown aseptically, was in direct proportion to the concentration of Williams' folic acid of potency 5000. This preparation gave half-maximal growth at levels of 0.002 γ per ml. of medium.

VI STUDIES OF FOLIC ACID IN HUMAN SUBJECTS

It is impossible in a review of this length to make a complete or comprehensive report on all of the earlier work in which substances were used in the treatment of anemia that might now be recognized as containing folic acid. To make the attempt would require that the brilliant demonstrations of the therapeutic effect of liver in pernicious anemia be included. Since liver therapy is so universally employed in medical practice, this aspect of the work is being omitted. We shall attempt, however, to give a background of thought and clinical experimentation which, combined with the evidence presented above, made the testing of *L. casei* factor in human beings seem an obvious step.

In 1931, Wills¹¹⁹ demonstrated the curative effect of a yeast extract in macrocytic anemia of pregnancy which occurs commonly in India. Some years later Wills and Evans¹²⁰ found cases of tropical macrocytic anemia which did not respond to the more highly purified liver extracts which were of therapeutic value in cases of pernicious anemia. This suggested the existence of a new hemopoietic factor in crude liver and autolyzed yeast extracts. This new factor could not be identified with thiamine, lactoflavin, or nicotinic acid but was present in a yeast fuller's earth filtrate. Wintrobe¹²¹ in 1939 reported maximal hemopoietic responses in five patients with pernicious anemia given dehydrated brewer's yeast at a level of 1-2 Gm. per Kg. body weight per day. In one case oral liver extract was more effective than the brewer's yeast. Nine patients on a maintenance level of 0.3-0.8 Gm. per Kg. body weight per day remained well for 4 to 10 months. Reticulocytosis occurred in two patients given yeast extracts parenterally but a significant increase in number of erythrocytes did not follow.

Several reports have appeared during the past few years concerning the anemia of pregnancy. Miller and Studdert¹²² made a study of 23 cases in which all were found to respond to specific therapy. Treatment could be discontinued in 14 of these cases. Dietary deficiencies and vomiting were important factors associated with the etiology of the anemia. Free hydrochloric acid was present in the gastric juice of 18 cases. In these, marmite plus a good mixed diet was usually sufficient treatment. In the remaining cases, where no free acid was present, or in those patients not responding to marmite, remission of the anemia followed injections of either a refined or crude liver extract. A degree of iron deficiency often became apparent during therapy. Davidson, Davis, and Innes¹²³ reported on 16 cases of anemia occurring during pregnancy which resembled Addisonian pernicious anemia. A megaloblastic bone marrow was found. Perseverance and intensive therapy in refractory cases was considered to be of vital importance. In three cases of macrocytic anemia of pregnancy and the puerperium, Fullerton¹²⁴ concluded that a deficiency of the antipernicious anemia principle was not the only factor concerned.

in the production of the anemia. Reticulocytosis following injections of liver extracts was not always followed by erythrocyte regeneration. Whole liver appeared to provide other factors necessary for restoring the blood to normal. He considered his cases analogous to the anemia associated with steatorrhea (see reference 138).

Anemia associated with deficiencies of the vitamin B complex has been studied by Moore, Minnich, Vilter, and Spies¹²⁵. Among 50 patients with hypochromic anemia, 32 had a clinical vitamin deficiency. All were found to respond satisfactorily to oral administration of ferrous salts. Brewer's yeast administered in some cases in doses of 25 Gm. three times daily did not alter the rate of increase of hemoglobin when compared to cases receiving iron but no yeast. Moore, Vilter, Minnich, and Spies¹²⁶ later reported that the oral or parenteral administration of a combination of niacin, thiamine, riboflavin, calcium pantothenate, pyridoxine, inositol, para-amino-benzoic acid, and choline had no effect in correcting the macrocytic anemia in patients with pellagra or deficiency of the vitamin B complex. This work was done on 25 patients with red blood cell counts under three million, whose diets had been deficient in animal protein and B vitamins for years. Clinical signs of vitamin deficiency such as glossitis, cheilosis, or peripheral neuritis were usually found. Nine of 10 patients gave a reticulocyte response to daily injections of 4-8 U.S.P. antipernicious anemia units in the form of highly purified liver extracts. Eight of these showed a marked acceleration in rate of increase in erythrocytes. A reticulocyte response followed the feeding of beef muscle and an 80 per cent alcoholic extract of beef in other cases. They felt that the anemia was due to a prolonged dietary deficiency of extrinsic factor often associated with poor absorption from the intestinal tract and possibly inadequate production of intrinsic factor by the gastric mucosa. Watson and Castle¹²⁷ reported the results of studies on three cases of nutritional anemia. All gave histories of dietary inadequacies, all had free hydrochloric acid, and two of the cases became anemic during pregnancy. The blood showed a macrocytic hyperchromic anemia with mild leukopenia and thrombocytopenia. Anisocytosis and poikilocytosis were less marked than in comparably severe pernicious anemia. The differential white cell count was normal. There were no neural manifestations. They found a prompt response to orally administered liver extracts immediately following therapeutic failure of liver extracts given parenterally, even in multiple U.S.P. units daily, in two cases. This showed a deficiency of some substance other than that effective in pernicious anemia. The third case indicated that intramuscular injections as a route for therapy were unsatisfactory in that ten times the normal amount was required.

A study was undertaken by Castle, Ross, Davidson, Burchenal, Fox, and Ham¹²⁸ to test additively the extrinsic factor activity of all members of the vitamin B complex and of certain other accessory nutritional factors. Casein was rendered free of extrinsic factor and administered simultaneously in the belief that one or more of the vitamins might become active as a prosthetic group on the casein molecule as a result of the action of gastric juice. They observed that the procedure required to convert crude casein into a vitamin free form was also essential for the elimination of the extrinsic factor and that extrinsic factor was not recon-

stituted when the vitamins were combined with the casein so treated, under the conditions and tests of this experiment. It was concluded, nevertheless, that extrinsic factor should still be regarded as an as yet unidentified thermostable component of the vitamin B complex.

Wright and Welch^{129, 130} measured by the *L. casei* growth method of Landy and Dickens¹² the daily urinary excretion of folic acid in 15 normal human subjects. The average value obtained from 42 samples was 0.0108 mgm. units. The average daily intake of folic acid in well fed adults is 1.4 mgm. units. This figure is derived from a report by Williams¹³¹ in which he states that a daily intake of 0.1 mgm. unit of folic acid is sufficient and the average figure was based on an assay of a well rounded mixed diet. When the urine samples were subjected to autoclaving with dilute acid or dilute alkali, or to digestion with takadiastase, no increase in the amount of folic acid was found. Incubation of the urine with a fresh rat liver preparation caused the appearance of more folic acid. The yield of the substance in urine which seems to be converted into the vitamin was increased by autoclaving for one and one-half hours in the presence of normal HCl. This treatment destroyed the free folic acid present. Since synthetic xanthopterin could be converted into folic acid under similar conditions, it was thought that possibly uropterin, said to be identical with xanthopterin, was the substance in urine capable of being enzymatically converted into folic acid. This reasoning would suggest that xanthopterin may constitute one part of the vitamin molecule. Johnson, Hamilton, and Mitchell¹³² verified the low urinary excretion of folic acid in normal human subjects reported by Wright and Welch. They found, however, that sweat contained five times as much folic acid as the urine when assayed with *L. casei* and about six times as much when *S. lactis* was the test organism. These values were obtained under conditions of profuse sweating and were on an hourly basis. They failed to increase the folic acid content of urine by incubation with vitamin B conjugase or takadiastase. The high ratio of excretion of folic acid in sweat when compared to urinary excretion is contrary to the values found with other members of the vitamin B complex.

In 1944, Sharp, Vonder Heide, and Wolters¹³³ reported the results of preliminary clinical studies on the antianemic action of vitamin B₁₂ in the form of a yeast concentrate. Ten patients, all of whom had been under observation for a year or more, were known to give no response to various types of antianemia therapy tested during this period. All had erythrocyte counts between 3.0 and 3.5 millions per cu mm. and 9-10 Gm. of hemoglobin per 100 cc. of blood. The vitamin B₁₂ concentrate was administered in amounts giving 600 γ per day, and after the first week it was increased to 1500 γ per day. At the end of four weeks treatment the hematocrit showed an appreciable increase, but otherwise there was little change in the blood picture.

Because of the results obtained with folic acid in the treatment of vitamin M deficiency in monkeys, it was considered worth while to test the effect of this substance on the leukopenia commonly seen in persons with a multiple vitamin B complex deficiency. A preliminary report by Berry, Spies, and Doan¹³⁴ indicated that either a folic acid concentrate from liver or the newly synthesized *L.*

cases factor when administered parenterally might elevate the total number of circulating leukocytes with a proportionate increase in granulocytes in some cases. There was a left shift in the Arneht nuclear index accompanying the rise, but the elevation was relatively transitory and in most cases was not maintained the following day. Of interest in this connection was a recent report by Watson, Sebrell, McKelvey, Daft, and Hawkinson⁴ in which elevations of leukocyte counts were obtained in each of 6 cases of leukopenia resulting from Roentgen ray therapy following oral administration of *L cases* factor concentrate. Five of the 6 cases were being irradiated for carcinoma of the cervix and the other for polycythemia vera. No effect was observed in a case of severe leukopenia following x-ray therapy for Hodgkins disease nor in 8 cases of refractory anemia.

Spies, Vilter, Koch, and Caldwell¹ also reported that a crude folic acid concentrate yielded no hematological response along with niacin, thiamine, riboflavin, calcium pantothenate, pyridoxine, inositol, para-amino-benzoic acid, choline, pyridoxamine, and pyridoxal in treating macrocytic anemia. They reported that materials of unknown structure isolated by Cline¹³⁵ from reticulogen, given parenterally to patients with macrocytic anemia, had produced reticulocyte responses of varying degree. However, in some cases there was no positive evidence of erythrocyte regeneration with 10-12 per cent reticulocytes, and in other cases a brief and minimal rise in reticulocytes was followed by an increase in erythrocytes, leukocytes, and hemoglobin. As Houghton and Doan¹³⁶ had previously emphasized, reticulocytosis alone is an insufficient index of erythrocyte maturation in the bioassay of potential hematopoietic substances. Peripheral blood counts, bone marrow differential counts, and plasma iron value may all be required. Spies et al. tested synthetic *L cases* factor on 5 cases of macrocytic anemia in relapse. The material was dissolved in saline made slightly alkaline with small amounts of NaHCO_3 and given parenterally. There was reticulocytosis and a slight increase in erythrocytes and hemoglobin in each case. Four additional macrocytic anemias were administered oral doses of *L cases* factor. All responded in a way comparable to that obtained following intravenous injection of the vitamin. The dosage ranged from 50 mgm. twice a day to 50 mgm. three times a day. A later report by Vilter, Spies, and Koch² was based on the study of 14 cases of macrocytic anemia. Six of these cases were diagnosed as nutritional macrocytic anemia, 5 cases were of Addisonian pernicious anemia, and 3 cases were of indeterminate types. All but 2 of the patients were hospitalized and their diets were closely regulated so that sources of extrinsic factor would be minimal. Only 1 patient received other vitamins concomitantly with the synthetic folic acid of the Lederle Laboratories. Thirteen of the 14 patients showed a positive hematological response consisting of reticulocytosis and subsequent rise in erythrocytes, hemoglobin, and leukocytes. In those cases in which treatment was continued the regeneration continued to normal levels (figs. 2 and 3). Erythrogenesis occurred regardless of the route of administration of the folic acid and regardless of the clinical classification of the macrocytic anemia. The responses were considered to parallel those afforded by liver extract.

Moore, Bierbaum, Welch, and Wright³ also obtained clinical and hematologic

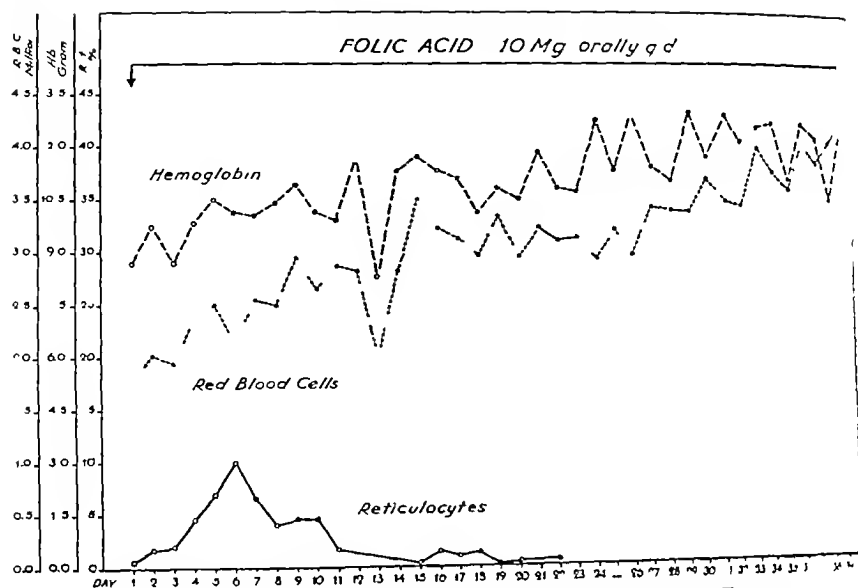


FIGURE 2. CASE OF NUTRITIONAL MACROCYTIC ANEMIA—FOLIC ACID THERAPY

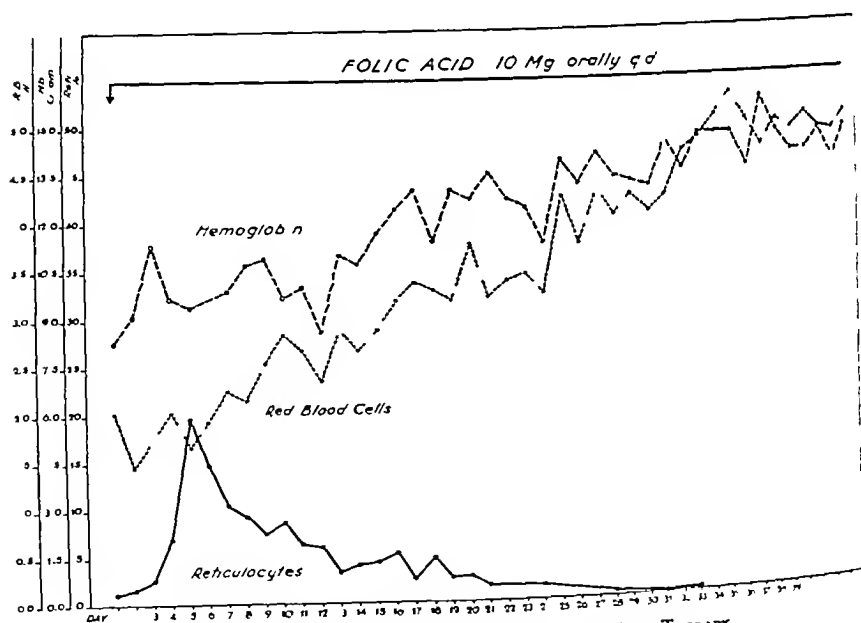


FIGURE 3. CASE OF PERNICIOUS ANEMIA—FOLIC ACID THERAPY

remissions in 2 patients with Addisonian pernicious anemia following the administration of synthetic *L* casei factor. One of these patients received a daily oral dose of 100 mg for 10 days. The initial red cell count rose from 1.2 million to 2.0 million beginning about the seventh day of therapy and was constant for 40

weeks. There were 40 per cent reticulocytes on the seventh day. The second patient started at 0.7-0.95 million, was transfused to 1.4-1.5 million, following which an oral dose of 30 mg folic acid daily for 14 days was given. There were 44.5 per cent reticulocytes on the eighth day and the red cells began to rise on the sixth, reaching 2.5 million on the fourteenth day. The leukopenia and thrombocytopenia were corrected. One case of nontropical sprue was given 20 mg *L. casesi* factor per day for 10 days and 40 mgm every two days for two weeks, all parenterally. The initial erythrocyte count of 2.6 million rose to 3.5 million and leveled off after 12 to 14 days. The maximum reticulocyte response of 30.2 per cent occurred on the seventh day. Leukocytes and platelets were normal within 7 to 10 days. A case of pernicious anemia of pregnancy was given 20 mgm folic acid parenterally daily for 10 days. The erythrocytes increased from 1.1 million to 3.0 million in 15 days and 48.2 per cent reticulocytes were found on the seventh day.

A preliminary report by Darby and Jones¹³⁷ on the treatment of nontropical sprue with synthetic *L. casesi* factor appeared late in 1945. One case was followed for fifteen days and the second for four days. Reticulocytosis occurred in both cases and the former showed an elevation in total number of erythrocytes. More recently, Darby, Jones, and Johnson⁵ have reported the treatment of three cases of sprue, all fulfilling the diagnostic criteria set up by Hanes¹³⁸ for this disease, with 15 mgm of synthetic *L. casesi* factor daily by the intramuscular route. The glossitis disappeared in 3 to 4 days followed by rapid regeneration of papillae. There was an improved sense of well-being, the diarrhea subsided, the appetite improved, and there was a decided gain in body weight. The maximum reticulocyte response was 15.3 per cent on the eighth day in one case, 43 per cent on the sixth day in another, and the third case had 11 per cent on the sixth day (day of publication). There was an increase in number of erythrocytes, grams of hemoglobin, platelets, and leukocytes. The sternal marrow returned to normal. At essentially the same time, Spies, Lopez, Menendez, Minnich, and Koch¹³⁹ reported from Cuba the preliminary results of oral administration of 100 mg synthetic folic acid twice daily to three selected cases of tropical sprue. The red blood cells ranged from 1.15 millions to 2.16 millions, the hemoglobin from 6.6 to 9.5 Gm, and after 10 days of therapy there was an elevation in both erythrocytes and hemoglobin. The maximum reticulocyte responses were 17.2, 17.2, and 22.7 per cent for these cases.

More extensive reports by Spies^{140, 141} definitely establish the therapeutic value of synthetic *L. casesi* factor in various clinical types of macrocytic anemia. Remissions were obtained in 5 cases of nutritional macrocytic anemia, 5 with Addisonian pernicious anemia, 8 with sprue (fig. 4), 3 macrocytic anemias of pregnancy, 1 macrocytic anemia associated with chronic alcoholism, cirrhosis of the liver, and neuritis, 1 macrocytic anemia associated with carcinoma of the stomach, and 3 of indeterminate etiology. No response was obtained in 3 cases of aplastic anemia, 3 cases of leukemia, and 4 cases of iron deficiency anemia. Dosages as large as 400 mg by mouth were given without ill effects. Five cases responded to 10 mg per day for ten days and all had previously failed to respond to doses of 5 mg per day for 10 days.

Spies, Milanes, Menendez, Koch, and Minnich¹⁴² extended the earlier observa-

tions on the therapeutic value of folic acid in tropical sprue. They emphasize the fact that folic acid should be considered an antianemic substance and that it is not necessarily identical with the antianemic substance(s) present in liver extract or dried brewer's yeast powder. In treating these cases of sprue the patient improved both clinically and hematologically while restricted to a diet devoid of meat and meat products. The authors do not recommend such a restriction under normal conditions of treatment but rather stress the importance of a high vitamin and high protein diet in hastening recovery and convalescence. Additional unpublished observations by Spies and his associates in Cuba and Puerto Rico indicate that folic acid has a profound effect on the alimentary tract of persons with sprue. Roentgenograms of the gastro-intestinal tract show that the highly irritated small bowel tends to become normal following *L. casei* factor therapy.

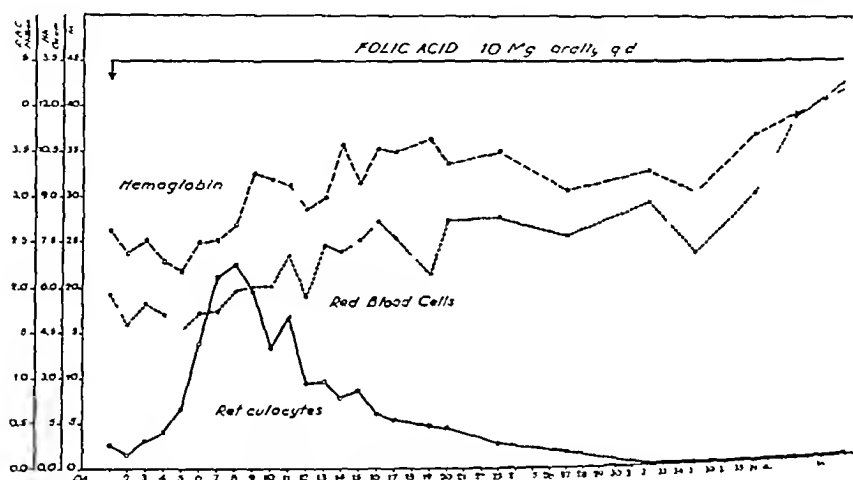
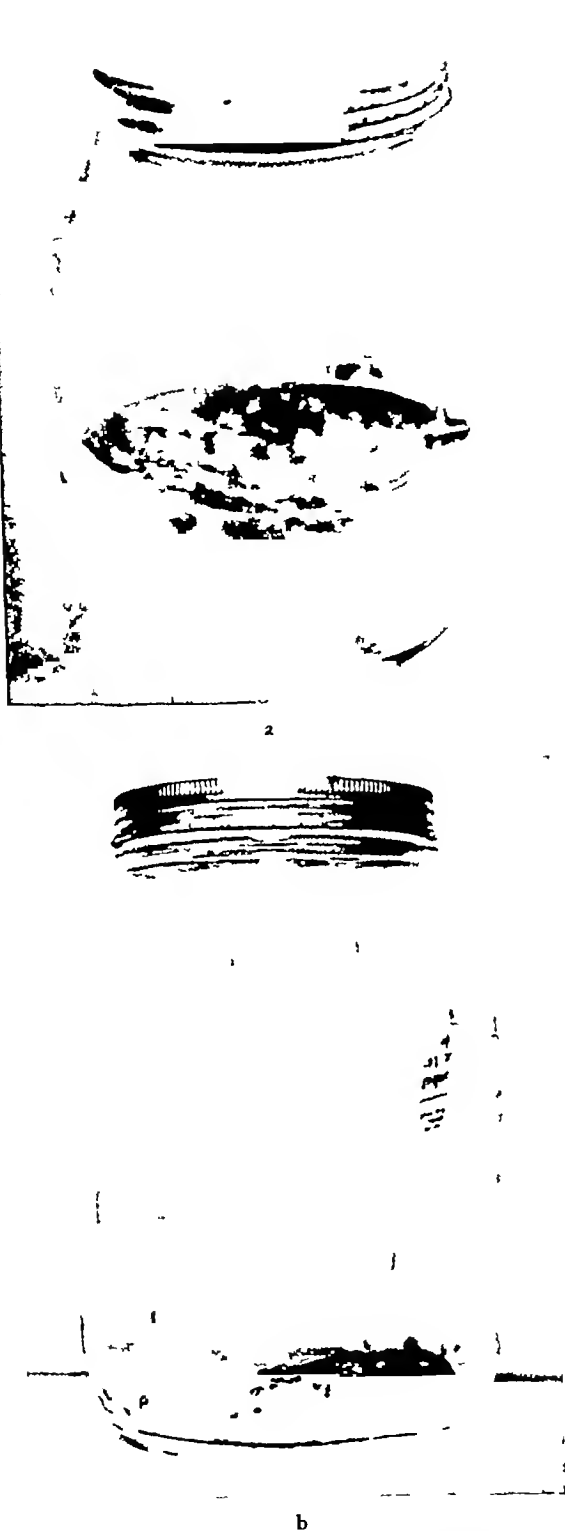


FIGURE 4 CASE OF SPRUE—FOLIC ACID THERAPY

There is also a marked improvement in the diarrhea. The number of stools per day decreases and the feces, loose and bulky before therapy, usually become formed and essentially normal in appearance (fig. 5). It has been found, however, that thiamine and niacin deficiencies may also contribute to the disorder. Frequently the red tongue of sprue can be relieved promptly by the administration of niacin alone. Folic acid has been found to produce a hemopoietic response in cases of tropical sprue simultaneously infected with intestinal parasites and even in the presence of severe pneumonia. These cases did not receive any specific treatment for the infections and hence responded to folic acid despite them.

Clinical evidence for the hemopoietic value of synthetic folic acid is rapidly accumulating. Moore, Bierbaum, Heinle, and Welch,¹⁴³ Goldsmith,¹⁴⁴ Doan, Wilson, and Wright,¹⁴⁵ and Vilter, Vilter, and Spies¹⁴⁶ All of these investigators have obtained remissions in all cases of macrocytic anemia. The smallest dose level so far reported is that of Doan et al., who obtained a complete remission in a case of Addisonian pernicious anemia with 2 mg. of folic acid given parenterally.



b

FIGURE 5 STOOL SAMPLES FROM A CASE OF TROPICAL SPRUE (a) BEFORE TREATMENT AND (b) FOLLOWING TREATMENT WITH SYNTHETIC *L. casei* FACTOR

daily for 20 days. The ability of this substance to maintain a normal blood picture is being investigated in various clinics. Until sufficient time has elapsed for more thorough testing, some doubt must remain as to the completeness with which folic acid can be expected to replace the standard liver therapy employed in such cases. However, Doan et al. and Spies have independently recommended folic acid for patients sensitive to liver extract and suggest that this is a safe and satisfactory procedure in such cases. Doan et al. also tested without success the value of folic acid in correcting the leukopenia of influenza and the pancytopenia of myelophthisic and idiopathic states.

The administration of the synthetic *L. casesi* factor to normal individuals has been found⁸ to produce no effect on the blood picture. Unpublished studies¹¹ on 18 college students and faculty members selected at random have been conducted over a period of several months. Each person was given 50 mgm. folic acid daily for two weeks. No change in the blood was observed except in those cases showing slight red cell deficiency. In every case the blood count was normal or slightly elevated at the end of administration. In 4 subjects the daily dose was continued for two to three months and the blood picture remained unchanged. These observations, along with others, suggest that folic acid has no undesirable action under the conditions of these studies.

These recent developments, therefore, offer bright hope for the long sought clue to the riddle of macrocytic anemia. Before too optimistic a view is taken, however, it might be wise to reflect on some of the questions which must be resolved concerning the place *L. casesi* factor occupies in this problem. As Moore et al.² and Spies et al.¹³⁹ point out, complete remissions have not as yet been obtained with the quantity of folic acid which Clark¹⁴⁸ has recently shown to be present in therapeutic doses of liver extract. Moreover, Subbarow, Hastings, and Elkins¹⁴⁹ in a recent review state that Loland, Klem, Strandell, and associates in Sweden have obtained hemopoietic responses with 0.7 mgm. of a liver fraction. From the description of the properties of this fraction, it seems unlikely that it is identical with (or even related to) the *L. casesi* factor. Subbarow et al. also, on the basis of their own experimentation, lean toward the view that there is no single antianemic principle in liver but rather that an interaction of as many as three different substances may be required. All of these factors may act in such small amounts that the chance for contamination seems unlikely. In any event, it seems safe to conclude that *L. casesi* factor is not an extrinsic factor since it acts orally in the absence of normal gastric juice and is also effective parenterally.

Recent observations by Spies, Vilter, Cline, and Frommeyer¹⁵⁰ add another contribution to the knowledge of antianemic substances. In one case of macrocytic hyperchromic anemia in relapse, with persistent histamine refractory achylia and achlorhydria, 2 Gm. of thymine were given orally thrice daily for 14 days. A peak reticulocytosis of 14.2 per cent was obtained on the eleventh day of treatment, and on the twentieth day after treatment the erythrocytes had increased from 2.01 millions to 2.74 millions and the hemoglobin had risen from 6.3 Gm. to 9.4 Gm. The bone marrow reverted from a megaloblastic to a normoblastic state. The same patient had previously failed to respond to 500 mgm. of thymine orally twice daily for 6 days. Spies, Frommeyer, Vilter, and English¹⁵¹ have since reported remissions

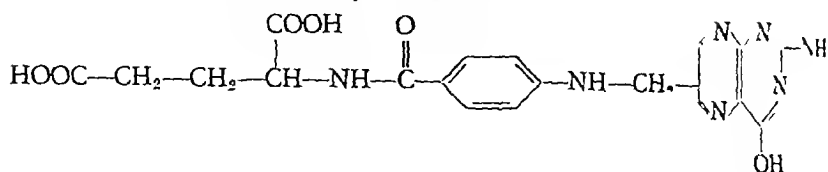
following large doses of thymine in three cases of Addisonian pernicious anemia in relapse. These patients were hospitalized and were given a diet devoid of meat or meat products. Doses up to 3.4 Gm. three times daily for 11 days were used. The clinical and hematological improvement was found to be similar to that which follows the administration of folic acid to patients with pernicious anemia in relapse.

Since thymine is part of the nucleic acid molecule, and since nucleic acids have long been known to play an important role in cellular metabolism, there is the suggestion that antianemic factors are linked to nucleic acid synthesis in some way. There seems to be little doubt but that liver extracts contain antianemic principles that are not identical with folic acid and could not possibly contain sufficient thymine. Thus Castle's erythrocyte maturation factor must either be the end product of the action of these several substances or else be several different substances capable of leading to the same end result. Should this end result be bound in with nucleic acid formation, it would seem, *a priori*, that folic acid and other antianemic factors may act in a similar over-all manner but possibly by different routes. Until more specific data are accumulated regarding the biochemistry of these compounds it would doubtless be wiser to omit further unfounded speculation. There is, however, every indication that the practicing physician will soon recognize in folic acid or thymine therapeutic tools of tremendous value in the handling of cases of macrocytic anemia in relapse. While the effect of thymine is of great scientific interest, it is of little practical importance since the dosage required is so very large, being somewhere in the neighborhood of 1200 times by weight the amount of folic acid required to produce a similar response.

Spies and associates have included pernicious anemia in the group of macrocytic anemias that respond to folic acid, despite the fact that they realize that its natural pathogenesis is somewhat different from the macrocytic anemia of sprue, pregnancy, pellagra, and from nutritional macrocytic anemia. Spies has considered that folic acid works as a part of an enzyme system and that it could be built up in the body and thus be made into an antipernicious anemia factor. His working hypothesis, however, is that folic acid in food occurs as a conjugate and that it is likely that in pernicious anemia the enzymes are unable to liberate efficiently the folic acid, whereas in persons with sprue, pellagra, pregnancy, and nutritional macrocytic anemia this substance and substances that act similarly are made more available.¹³⁹ He¹⁴⁰ and Moore³ have consistently stressed that a satisfactory explanation has not been made concerning the fact that a large amount of folic acid is required to produce a satisfactory hemopoietic response in contrast to a highly potent liver extract in which the active substance is smaller. This naturally leads to the thought that perhaps the folic acid conjugates are stored in our bodies and that liver extract may contain a substance capable of liberating from them the substance which acts on the bone marrow.

Today at least four crystalline compounds have been obtained from liver, yeast, and other sources. These compounds have somewhat different properties, and soon their relationship one to the other will be greatly clarified, since the chemical structure is becoming known.

The liver *L. casesi* factor has been isolated and synthesized, and the formula which is as follows, has recently been announced¹⁵



N-[4-[(2-Amino-4-hydroxy-6-pteridyl) methyl]amino]benzoyl]glutamic Acid

The fermentation *L. casesi* factor differs in the number of molecules of glutamic acid, as they both contain the same heterocyclic ring structure. Perhaps the term pteroylglutamic acid will be a suitable chemical name for the liver *L. casesi* factor.

Spies, in unpublished observations, has had cases of Addisonian pernicious anemia, nutritional macrocytic anemia, and sprue which have given complete remission and have developed normal blood values. In those cases which did not develop entirely normal blood values, synthetic vitamins and liver extract were tried independently and together and in no instance produced any augmentation.

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TREATMENT OF LIVER EXTRACT SENSITIVITY

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WITH the rapidly increasing use of parenteral liver extract, the therapeutic problem of the development of sensitivity to liver extract has become increasingly common. That this problem is frequent enough to be of practical importance is illustrated by the fact that 68 of 396 out-patients (17 per cent) having pernicious anemia and receiving liver extract injections developed manifestations of sensitivity during the period of this study from 1940 through 1945.*

The first report of sensitivity to liver extract was that of Schlesinger in 1930.¹² In 1943 Kaufman, Farmer, and Reich⁶ collected 35 reports from the literature dealing with 50 cases and added 11 more. Large series were concurrently reported by Barfred¹ (19), Feinberg, Alt, and Young⁴ (8), Delikat³ (14), and others.† Since with prolonged therapy the opportunity for sensitization increases and since more and more patients are receiving liver injections, those becoming sensitized will almost certainly increase in number.

The types of reactions encountered in our cases were similar to those previously described and varied from itching, slight flushing, tachycardia, cough, nasal and ocular discharges, and localized urticaria to generalized urticaria, weakness, faintness, nausea, vomiting, broncho-spasm, asthmatic reaction, substernal pain, collapse, rigor, profound shock, and rarely death. It is interesting to note in this connection that the only fatal reaction reported in the literature is that of Morgans,¹⁰ implying that reactions of extreme severity are practically unknown. However, we know of at least one case in which an injection of liver extract was followed by death. Of incidental interest in this case was the fact that the patient was given liver extract for an anemia not of the type characteristically responding to liver. In addition, we have seen three reactions in patients of known sensitivity, to whom liver extract was inadvertently given in large dosage. Had it not been for the immediate injection of epinephrine solution, death might conceivably have ensued. The first symptom of sensitivity occurred at any time during the course of therapy, and occasionally with the first injection. In some cases no symptoms were noted until several years after the commencement of therapy. As a rule the reactions with each succeeding injection became progressively more severe though occasionally they ceased spontaneously after one or even two or three episodes.

There is no universal agreement as to the cause of sensitization to liver extract. Ryne and Tocantins¹¹ felt that the sensitization in their case was that of a biologically specific type. Their patient was very sensitive to liver but most especially pork liver and pork tissue. On the other hand, Feinberg, Alt, and Young⁴ found positive skin tests with pork, beef, and horse liver. They did not feel that the

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The Antigen H used in this study was furnished by the Parke, Davis & Co. Laboratories.

* During this same period the senior author observed the same phenomenon in 5 private patients.

† To these must be added the singular cases, of which the authors hear continually, but which are never reported. Publication of these would serve to emphasize the commonness of the experience.

protein of the liver was the sensitizing antigen, but rather that the anti pernicious anemia principle was in itself antigenic and therefore was neither species nor organ specific. Kaufman, Farmer, and Reich⁶ felt that the brand of the liver material made no difference, thereby implying that the different animal sources would also be of little significance. Our own clinical observations are in disagreement with both Fernberg's and Kaufman's conclusions regarding the specific sensitivity to the anti pernicious principle and the lack of difference in brands of liver. Although a considerable disagreement exists as to the fundamental cause, there is general agreement that the reaction differs in no way from ordinary foreign protein sensitization and that the type of reaction seen in liver extract sensitivity has, in common with other allergic responses, the symptoms caused by tissue reaction to histamine or the so-called H substance.

TREATMENT OF THE PATIENTS WITH REACTIONS

It is obvious that with so many reactions the problem of treatment becomes very important. Numerous suggestions for the treatment of liver extract sensitivity

TABLE 1—Summary of Cases with Reactions to Liver Extract and Therapy

Cases	Treatment	Result
3	No therapy	No recurrence of reactions
1	Reduction of dose	Tolerance to 2 cc. dose (regular dose 3 cc.)
7	Changed to crude liver extract	No recurrence of reactions
14	Changed brand of liver extract	No recurrence of reactions
9	Changed brand of liver extract	Recurrent reactions
24	Simple desensitization	No recurrence of reactions
5	Repeated simple desensitization	No recurrence of reactions
3	Simple desensitization	Recurrent reactions
9	Data incomplete	Disappeared from observation
10	Antigen H	Disappearance of reactions
1	Antigen H	Tolerating small doses

have been made (table 1). Lasch⁸ suggested calcium, orally and parenterally, together with the administration of parathyroid to raise the blood calcium. Corelli² proposed the administration of increasing doses of histamine to produce histamine refractoriness. Histaminase was suggested by Taylor and Hilger¹¹ to inactivate histamine. Grün,⁵ Krantz,⁷ Delikat,³ McSorley,⁹ and many others proposed desensitization by progressively increasing doses of liver extract.

It is seldom that one is immediately aware of the first reaction, since the patient has left the clinic by the time the untoward symptoms manifest themselves. Very often the patient fails to mention that anything unusual has happened until he has

* We have not used the technic of desensitization advocated by McSorley and Davidson but their results are apparently not dissimilar to our own. We disagree however, with their conclusions that reactions tend to recur if injections are spaced at several week intervals and their administration intervals between injections should on no account exceed four weeks. In analyzing our figures we found no statistical difference in the incidence of reactions in the groups of patients who received their injection at intervals varying from one to six weeks.

had several increasingly severe reactions. When the patient comments that he had flushing or itching after the previous injection, it has been our first procedure to reduce the dose of liver. The customary dose of liver extract in our clinic has been 3 cc of a liver extract of 10 units per cc potency and, depending on the patient's need, varying the time interval between injections. With the history of a previous reaction, the dose is reduced to 1 cc, and if no reaction occurs 0.5 cc is added at each subsequent visit until the full dose of 3 cc is again reached. During this time the interval between doses is reduced from the usual one of two to six weeks to one week. If, however, the 1 cc dose still elicits a reaction, the dose is reduced to 0.1 cc.* In this case, the dose is again increased by 0.1 cc weekly until 1 cc is reached. If this is well tolerated, the increment becomes 0.2 cc until 2 cc is reached, then 0.5 cc to 3 cc. Once the full maintenance dose is again reached the intervals are once more gradually lengthened, depending upon the patient's needs. By this simple procedure we have been able to desensitize 24 patients.

In 3 cases, the patients failed to mention a previous reaction until after the subsequent full dose was given. In none of these cases was the second injection followed by any untoward symptoms, and it must be assumed that the reactions were of a minor nature or possibly due to the batch of liver used. How often episodes of this type may have occurred without our knowledge is impossible to estimate but is probably quite high.

In one case, it was possible to desensitize the patient and maintain him on 2 cc doses, but any attempts to increase this resulted in allergic reactions.

Nine patients in the present series and the 5 private ones were changed to other brands of liver extract without recurrence of reactions (beef plus pork to beef). It must be emphasized in this connection that when the brand of liver is changed the new liver extract should also be given in reduced dosage. Our custom has been to begin with 1 cc of a 10-15 unit liver extract and increase this by 0.5 cc increments until the full dose is reached. One of our cases illustrates the importance of observing this precaution. This patient was originally treated with a certain liver extract. Subsequently he was maintained for several years on a different brand. When he was inadvertently changed back to the original liver he developed a profound shock-like reaction. He had apparently been sensitized by the first liver and manifested severe sensitivity when this was again used several years later.

An occasional patient sensitive to purified forms of extract appears to tolerate cruder ones (2 units per cc of the same manufacturer) without reaction. This would support the opinion of Feinberg et al. that the sensitization is to the liver extract principle itself. There were 7 of these in our group. Eventually some of these patients again tolerated purified extracts, while others remained sensitive to them.

In 9 cases, though repeated attempts were made to change the brand of liver, reactions recurred.

In 5 it was possible to desensitize with the same extract by repeated attempts.

* For the immediate treatment of a reaction we use epinephrine solution 1:1000 in doses of 0.5 cc to 1.0 cc subcutaneously. It is one of the inflexible rules of the clinic that epinephrine solution is always at hand and available immediately in case of a reaction.

In these cases there was a recurrence of reactions upon reaching a certain amount, whereupon the dose was reduced and again gradually increased. Ultimately, all 5 became completely desensitized and had no subsequent recurrences.

In 3 cases the desensitization was completely unsuccessful. In none of these was the attempt made to desensitize with Antigen H, since these patients were unable or unwilling to cooperate.* One of these patients developed a sensitivity not only to parenteral and oral liver extract but even to small amounts of oral liver. She was ultimately poorly maintained on inadequate doses of stomach preparations.

Nine additional patients manifested sensitivity but their records are incomplete. They disappeared after either the first relatively serious reaction or soon after desensitization was begun.

There were 11 patients in whom the above described maneuvers to reestablish adequate levels of parenteral liver therapy failed. These were treated with Antigen H and, with one exception, were successfully desensitized. They represented the most difficult cases and posed not only a therapeutic challenge but a practical problem of prime importance, since a number of them had neurologic changes for whose alleviation parenteral therapy may be said to be mandatory. In addition it was economically impractical to maintain them on oral liver or stomach extract. All but one of these cases are now tolerating full maintenance doses, though some are still receiving Antigen H. The one exception now tolerates larger amounts of liver and is receiving adequate doses for maintenance.†

NATURE OF ANTIGEN H

Antigen H is para-amino-benzoyl histamine coupled with a protein.‡ It was prepared as an antigenic complex which could engender antibodies whose specificity would be determined by the histamine portion of the molecule. The antibody thus formed would act as a neutralizer for the histamine or H substance released by the tissues in response to sensitizing substances. It thus seemed logical to use Antigen H in the liver extract sensitive patient, whose sensitivity ultimately depended on the liberation of histamine or the H substance.

In the 10 patients (tables 2, 3, and 4) whose liver extract sensitivity did not respond to previously discussed means of treatment, Antigen H was injected subcutaneously in doses starting with 0.1 cc along with the maximal tolerated dose of liver. The two substances were injected separately, the liver being given intramuscularly. The Antigen H was increased by 0.1 cc weekly until the patient was receiving 1.0 cc. Concurrently the dose of the liver was also increased, and it was found in most instances that the patients' tolerance to it was rapidly enhanced. The Antigen H administration was discontinued in some cases when

* One of these patients became extremely allergic to parenteral liver and had to be maintained on oral liver extract. Two years ago he disappeared from observation and has just reentered the hospital in relapse. At the time of this writing he is tolerating full doses of liver extract without any allergic manifestations.

† It will be noted that the above cases do not add up to 68. This is because some patients had more than one episode of sensitivity and were variously treated.

‡ Parke, Davis and Co.

TABLE 2.—*Reactions to Liver Extract Treated with Histamine H*
Case 9 Curtis G, 42 Yrs, Colored Male

Date	Dose	Brand of Liver	Remarks
1/7/41 to 1/19/41	0 3 cc.	Assay liver (Wilson)	
1/20	0 3 cc.		Reaction
1/21	3 0 cc	Wilson (10 unit)	Reaction
1/30	0 5 cc		
2/6	1 0 cc		Reaction
2/13	1 0 cc	Campolon	
2/20	2 0 cc		
2/27	3 0 cc		
3/6	4 0 cc		
3/12	3 0 cc	Wilson (10 unit)	Reaction (collapse, syncope, shock)
3/27/41 to 11/22/44		Oral liver extract (Wilson)	
11/22/44	0 1 cc	Wilson (10 unit)	Antigen H 0 1 cc
11/30	0 2 cc.	(moderate reaction)	0 1 cc.
12/7	0 1 cc		0 3 cc
12/14	0 1 cc		0 4 cc
12/21	0 2 cc		0 5 cc.
12/28	0 3 cc		0 6 cc
1/ 4/45	0 4 cc		0 7 cc
1/17	0 5 cc		0 8 cc.
1/24	0 6 cc.		0 9 cc.
2/1	0 7 cc		1 0 cc.
2/8	0 8 cc.		1 0 cc
2/15	0 9 cc		1 0 cc
2/22	1 0 cc		1 0 cc.
3/1 /45	1 1 cc.		1 0 cc
3/15	1 2 cc.		1 0 cc.
3/22	1 3 cc		1 0 cc
3/29	1 4 cc		1 0 cc.
4/5	1 5 cc		1 0 cc
4/19	1 6 cc		1 0 cc
4/26	1 7 cc		1 0 cc.
5/3	1 8 cc		1 0 cc.
5/17	2 0 cc		1 0 cc.
5/24	2 2 cc.		1 0 cc.
5/31	2 6 cc.		1 0 cc
6/7	3 0 cc.		1 0 cc
			(Itching tightness in throat)
6/14	3 0 cc.		Antigen H 1 0 cc.
6/21	3 0 cc		1 0 cc.
7/5	3 0 cc		1 0 cc.
7/16	3 0 cc.		1 0 cc
9/12	3 0 cc.		1 0 cc
10/3	3 0 cc		
10/11	3 0 cc.		
11/14	3 0 cc		
11/31	3 0 cc.		
12/16	3 0 cc.		
1/16/46 to 5/1/46	3 0 cc.		

TABLE 3—*Reactions to Liver Extract Treated with Histamine H*
Case 10 Elizabeth W, 34 Yrs, White Female

Date	Dose	Brand of Liver	Remarks
4/6 /39	5 0 cc	Lilly	
4/13	2 0 cc.	Armour	
4/20	3 0 cc	Lederle	
4/27	3 3 cc	Lederle	
5/4	3 3 cc	Lederle	
6/1	3 3 cc.	Lederle	Reaction
7/6	2 0 cc.	Wilson	Reaction
8/3	1 0 cc	Lilly	
9/7	1 0 cc.	Lilly	
10/12	1 0 cc	Lilly	Reaction
10/19	0 2 cc.	Wilson	
10/27	0 4 cc	Wilson	Reaction
10/27/39	oral liver and liver extract		
7/3 /41			
7/3 /41	0 0 cc	Wilson (10 unit)	
7/7	0 2 cc.		
7/8	0 3 cc		
7/9	0 4 cc.		
7/10	0 5 cc		
7/11	0 6 cc.		
7/12	0 7 cc		
7/14	0 8 cc.		
7/15	0 9 cc.		
7/16	1 0 cc.		Local tenderness and swelling
7/17	0 5 cc.		
7/18	0 6 cc		
7/19	0 7 cc.		
7/21	0 8 cc		
7/24	0 9 cc		
7/26	1 0 cc.		Itching face and hands
7/31	0 5 cc.		
8/7	0 75 cc		Itching, redness hands
8/14	0 5 cc.		
8/21	0 75 cc.		
8/28	1 0 cc.		
9/4	1 5 cc		
10/23	1 5 cc.		
11/6	2 0 cc		
3/5 /42	2 0 cc.		
3/12	2 0 cc		
3/26	2 0 cc.		Itching
4/9	1 0 cc.		
4/23	1 5 cc		
5/6	1 7 cc.		Itching headache, nausea
5/21	0 5 cc.		
6/4	0 7 cc.		
6/18	0 8 cc.		Slight reaction
6/25	0 7 cc.	(crude 2 unit)	
7/2	1 0 cc		

TABLE 3 —*Concluded*

Date	Dose	Brand of Liver	Remarks
7/9	1 0 cc	Wilson (crude, 2 unit)	
7/16	1 2 cc		
7/23	1 5 cc.		
7/30	2 0 cc		
8/6	2 5 cc		
8/27			
19 injections to 4/8/43	3 0 cc.		
4/15/43	3 0 cc		Antigen H 0 1 cc
4/22	3 0 cc		0 2 cc
4/29	3 0 cc		0 3 cc
5/6	3 0 cc		0 4 cc.
5/13	3 0 cc.		0 5 cc.
5/20	3 0 cc.		0 5 cc
5/27	3 0 cc		0 6 cc
6/3	3 0 cc.		0 8 cc
6/10	3 0 cc		1 0 cc
7/1	3 0 cc.		
7/15	3 0 cc.		
8/18	3 0 cc.		
9/16	3 0 cc		Itching palms and redness in face
9/27	0 5 cc.	(crude, 2 unit)	
10/7	1 0 cc.		
10/27	1 5 cc.	Antigen H 0 5 cc	
11/4	2 0 cc	0 6 cc.	
11/11	2 5 cc.	0 7 cc	
12/2	3 0 cc.	0 8 cc	
1/6 /44	3 0 cc	0 9 cc.	
2/3	3 0 cc	1 0 cc	
2/17	3 0 cc		
2/24	3 0 cc	(crude, 10 unit)	
3/22	0 5 cc		
4/13	1 0 cc		
4/27	2 0 cc.		
5/11/44	3 0 cc		
1/3/46	Injections of 3 0 cc. Wilson (10 unit)		
to 5/2/46			

it reached 1 0 cc. In some, tolerance to full doses of liver persisted without further administration of Antigen H, whereas in others there again developed various degrees of sensitivity which necessitated the readministration of the material.

Evaluation of therapy of this nature is extremely difficult and must be interpreted with caution since spontaneous loss of sensitivity is so common. However, the fact that the cases utilized for this series were all found to be extremely sensitive and had been extensively studied previously, plus the fact that we were able to get consistently rather good results, makes us believe that the Antigen H was of value. Notwithstanding this general impression it is noteworthy that the analysis of any single case might make one doubtful of the validity of these conclusions.

TABLE 4—Summary of Cases with Liver Extract Sensitivity Treated with Antigen H

Name	Total time from start of therapy to first reaction	Intervals during which patient received no therapy during initial period	Interval after resumption of therapy before reaction developed	Type of reaction	Treatment	Reaction free interval on full doses of liver	Type of reaction	Treatment	Reaction free interval on full doses of liver	Type of reaction	Treatment	Reaction free interval on full doses of liver
1 John McT	40 mos	24 mos	14 mos	Redness, itching, urticaria	'crude' liver, 'Anti gen H'	11 mos			then disappeared			
2 Kathryn F	6 mos	none		Redness, itching, fainting, urticaria	'crude' liver, 'Anti gen H'	5 mos		to present time	21 mos			
3 Catherine G	10 mos	none		Redness, itching, urticaria	crude liver, 'Anti gen H'	8 mos			disappeared			
4 Charles C	1 mo	none		Headache, vertigo, vomiting, urticaria	simple desensitization	2½ mos	Sick—not aware of self	to present time	34 mos			
5 Beila C	14 mos	4 mos	3 mos	itching, urticaria	Increasing doses of crude liver	12 mos	Eyes watering, lacrimation	to present time	24 mos			

[illegible]

No untoward results were noted with the Antigen H with the exception of an occasional small tender mass which persisted for some days at the site of the injection

With the promise of synthetic preparations which may eventually replace liver therapy (oral folic acid) and with the encouraging reports on oral anti histamin preparations the problems herein discussed may take their place with other antiquated medical questions which at the time of their practical challenge are quite baffling but in retrospect completely lose their value and significance

SUMMARY

Sensitivity reactions to the parenteral administration of liver extract are not uncommon. They were present in 68 of 396 patients (17 per cent) receiving liver therapy

The symptoms of sensitivity to liver extract are those of any foreign protein reaction

Various technics can be used to circumvent reactions and still maintain patients on adequate doses of liver extract

These methods are

A Changing the brand or type of liver extract

B Reducing the dose of liver extract

C Desensitization with liver extract

D Utilizing a histamine-protein complex as an antigen to produce histamine antibodies

The latter method was successful in 10 of 11 cases in which other methods had been without effect

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RUPTURE OF THE SPLEEN IN INFECTIOUS MONONUCLEOSIS

A CLINICOPATHOLOGIC REPORT OF SEVEN CASES

By MAJOR E B SMITH, M C , AND LT COL R P CUSTER, M C

INFECTIOUS mononucleosis is properly regarded as a benign disease. When death occurs it can be ascribed to a complication rather than to the disease itself. Pneumonia, edema of the glottis, and hemorrhage from a deep tonsillar ulceration were terminal conditions in a few cases of which we have personal knowledge. Rupture of the spleen has received little attention as a possible complication of infectious mononucleosis as is evidenced by the fact that only 3 well established cases have been reported in the literature (Darley et al,³ King,¹ Ziegler²). In 1922, before the disease was regarded as an entity, Friesleben⁴ described spontaneous rupture of the spleen in a case that was probably infectious mononucleosis. The seriousness of splenic rupture in infectious mononucleosis is emphasized by the 4 deaths in our series of 7 cases, as well as in Ziegler's case. Splenectomy resulted in cure of the others.

That infectious mononucleosis is a not uncommon cause of splenic rupture is indicated by the relative incidence of the condition in the files of the Army Institute of Pathology. Of 44 cases coded as Spleen, spontaneous rupture, infectious mononucleosis stood second as a cause in 7, exceeded only by recurrent malaria in 22 (table 1).

REPORT OF CASES

The 7 cases to be described are represented by specimens and records at the Army Institute of Pathology, 6 having occurred in military personnel during World War II, the seventh having been contributed by a civilian hospital. The diagnosis of infectious mononucleosis was made by careful examination and comparison of the pathologic material and the histories, the striking constancy of the histopathologic picture in the spleen served to substantiate our opinion that the diagnosis in each case was valid.

It will be noted in several cases that rupture of the spleen is followed by a reversal of the differential formula of the leukocytes, with neutrophils predominating. This may prove confusing unless one considers two factors: first, hemorrhage into a serous cavity is almost invariably followed by a striking neutrophilic leukocytosis and, second, the bone marrow in infectious mononucleosis is normal and fully capable of responding to this stimulus to the point of overshadowing the pre-existing abnormal lymphocytosis.

Case 1 (A I P No 103388) A white officer aged 21 years entered the Station Hospital, Camp Irwin, on November 4, 1943, with primary complaints of constipation and malaise. He had not felt up to par for

From the Army Institute of Pathology, Washington, D C

TABLE I

Case	AIP No	Blurred Pattern	Cellular Infiltration				Follicles			Sinuses		Cells							Spleen Weight	Diagnosis
			Arteries	Veins	Endothelium	Capsule	Few	Small	Large	Dilated	Swollen Endothelium	Abnormal Lymphocytes	Neutrophils	Erythrocytes	Plant Cells	Plasma	Diagnocytes	Plasma		
King et al		+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	665	Infectious Mononucleosis
Ziegler		+	-	-	-	+	+	+	-	-	-	+	+	-	-	-	+	-	3x	
Darley et al		+	-	-	-	-	+	+	-	-	+	+	-	-	+	-	+	-	460	
1	103388	+	+	+	+	+	-	-	-	-	+	+	-	-	-	-	-	-	470	
2	102434	+	+	+	+	-	+	-	-	-	+	+	-	-	-	-	-	-	494	
3	149279	±	+	+	+	+	+	-	-	-	+	+	-	-	-	-	-	-	4x	
4	100124	+	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-	•	
5	144648	+	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-	425	
6	94425	+	+	+	+	+	+	+	-	-	+	+	-	-	-	-	-	-	4x	
7	67260	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	?	
8	133724	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	500	Malaria
9	1530-5	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	+	750	
10	130836	-	-	-	+	-	+	+	-	-	-	-	+	-	-	-	+	+	510	
11	144235	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	660	
12	131093	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	+	675	
13	133015	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	+	•	
14	125710	±	-	-	-	-	+	+	-	-	-	-	-	-	-	-	+	+	385	
15	25435	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	+	+	•	
16	148944	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	+	666	
17	145512	-	-	-	-	-	+	+	+	+	+	-	-	-	-	-	+	+	•	
18	47351	+	-	+	-	-	+	-	+	-	+	-	+	-	-	-	+	+	500	
19	53405	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	700	Bantus Syndrome
20	93606	-	-	-	-	-	+	-	-	+	+	-	-	-	-	-	+	-	600	
21	160-80	-	-	-	-	-	+	-	+	+	+	-	-	-	-	-	+	-	940	
22	107248	-	-	-	-	-	+	-	-	+	+	-	+	-	-	-	+	-	1,100	
23	141054	±	-	-	-	-	+	+	-	+	+	-	-	-	-	-	+	-	1,100	
24	139321	±	+	-	+	-	+	-	+	-	-	-	-	-	-	-	+	-	300	Leukemia
25	91203	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	300	
26	138-45	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	440	Leukemia
27	89516	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	700	
28	71555	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1,100	
29	152011	+	-	-	+	-	+	+	-	-	-	-	-	-	-	-	+	-	200	Unknown
30	110071	-	-	-	±	+	+	+	-	+	-	-	+	-	-	-	-	-	500	
31	62101	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	300	
32	137103	-	-	-	-	-	-	+	-	-	+	-	-	+	+	-	-	-	300	
33	89901	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-	15	

* Spleen weight not given, but organ noted to be enlarged

† No material for examination in 11 additional cases of chronic malaria

3 weeks and thought that he had had bouts of fever. Two days before admission he had been given mineral oil and cascara at his Battalion Dispensary without relief from constipation, and the following day had transient sharp abdominal pain, but continued on duty. The day of admission he vomited several times and experienced a few severe cramps. Physical examination disclosed no abnormalities apart from moist rales and poor heart sounds, blood pressure was 90/40 mm Hg. Although the patient slept well and his color and the quality of his pulse improved, he became extremely dyspneic when lying on the back or right side, and moderate tenderness was elicited over the splenic region. During the day of November 5 tympanites developed and the blood pressure fell again to 80/60 mm Hg. At operation about 2 liters of partially clotted blood was removed along with the spleen, which weighed 470 Gm. The removed organ was soft and friable, and presented several bleeding lacerations of the capsule.

Laboratory Data

	R B C (M)	Hb (%)	W B C (Thousands)	Neutro (%)	Lympho (%)	Mono (%)
Nov 5	3 68		14 4	84	16	0
Nov 9	3 36	75	22 0			

Heterophil antibody titration was not done. Urinalyses were negative.

The day following splenectomy the patient's temperature rose to 105° F and he developed a cough productive of mucopurulent material. The diagnosis of pneumonia was made, the patient placed in an oxygen tent and sulfonamide therapy instituted. On the tenth postoperative day separation of the apparently healed wound occurred and was repaired under intravenous anesthesia. The patient died the following day. At autopsy the immediate cause of death was found to be pulmonary arterial thrombosis.

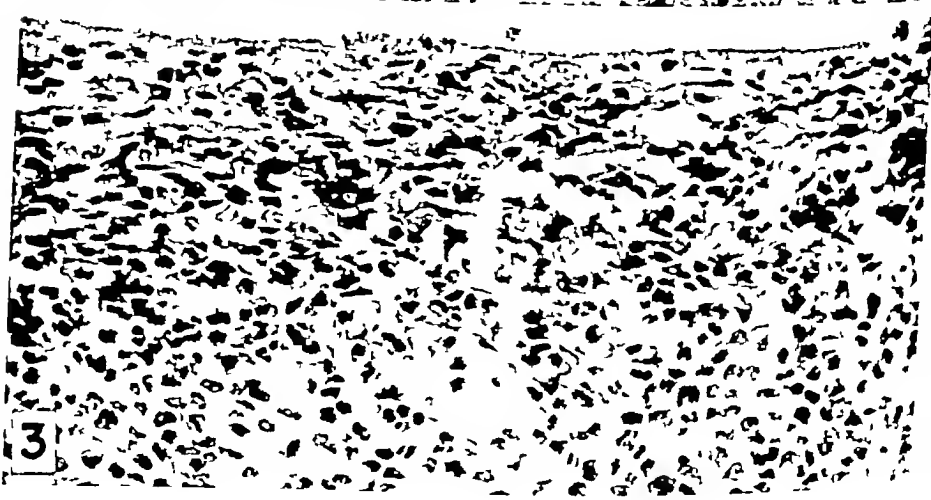
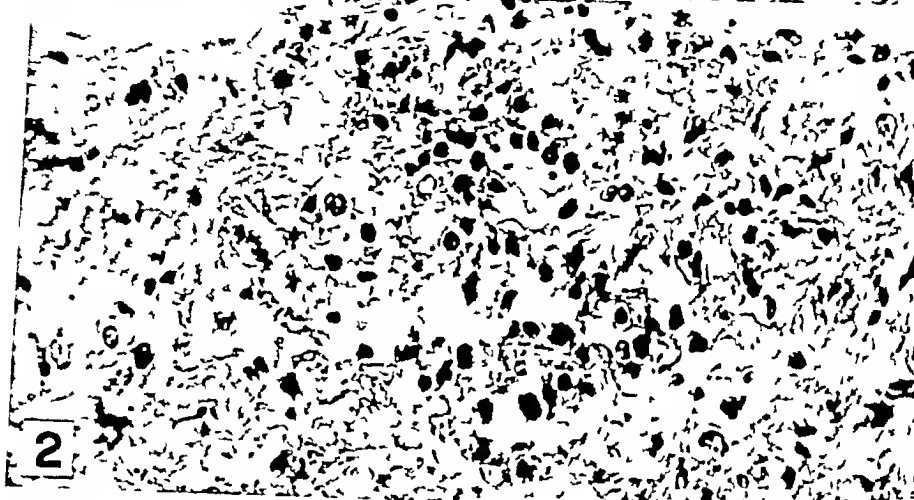
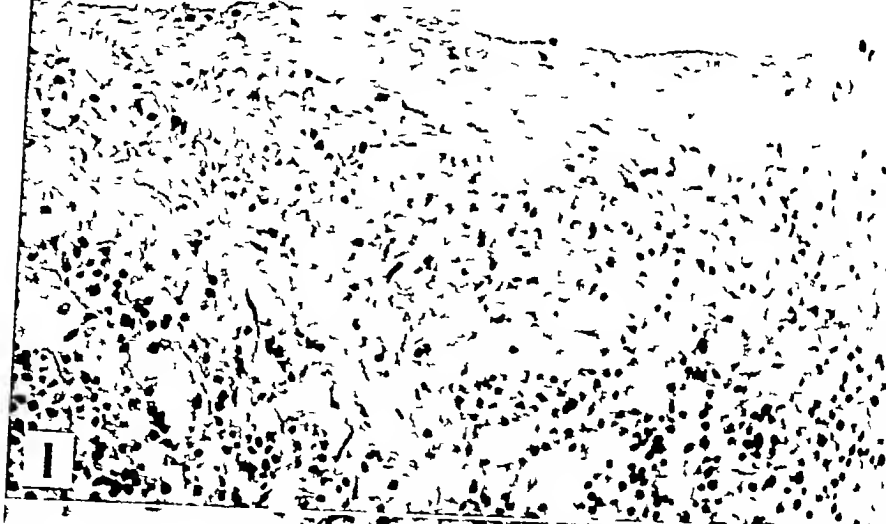
The diagnosis of infectious mononucleosis is strongly suggested by the history of an illness characterized by malaise and fever of 3 weeks' duration, mild enough that the patient could remain on duty, but productive of an enlarged spleen which ruptured. This impression is strengthened by the presence of histologic changes in the spleen and other tissues identical with those found consistently in proven cases of this disease.

Case 2 (A I P No 102434) A young officer of unstated age was admitted to the 2nd Evacuation Hospital on July 27, 1943, with a history suggestive of infectious mononucleosis of 15 days' duration. His blood smear contained atypical lymphoid cells warranting a tentative diagnosis of that disease. The same evening the patient suffered intense abdominal pain with associated spasm and rigidity. Splenectomy was performed the next day and a small piece of liver removed for biopsy; clotted blood was found in the peritoneal cavity. The spleen weighed 494 Gm, measured 18 x 12 x 3.5 cm, and its capsule was rent at the lower pole.

Laboratory Data

	Leukocytes Majority atypical lymphocytes	Heterophil Agglutination
July 27		1 80
July 28		1 80
July 30	11,450	
August 10		1 40
August 20		1 20
August 30	4,200	

Recovery was uneventful. The diagnosis of infectious mononucleosis was based on the clinical course, abnormal blood picture, and falling heterophil antibody titer during the recovery phase.



Case 3 (A I P No 149279) A white soldier of 24 reported to his Battalion Dispensary at 8 45 A M, February 8, 1945, with complaints suggestive of acute gastritis. He was referred to the 38th Evacuation Hospital with the tentative diagnosis of acute hepatitis without jaundice. On admission at 10 55 A M his condition was regarded as good. He was dead at 11 30 A M.

The peritoneal cavity contained 4 liters of partially clotted blood. The spleen was enlarged to four times normal size and showed extensive areas of laceration with the splenic pulp bulging out. The parenchyma was soft friable, and gray purple; the splenic artery and vein were normal. Peyer's patches were slightly hypertrophic, but no lymph node enlargement was noted. Other organs appeared normal on gross inspection. Malarial parasites were absent in smears, and cultures of feces, spleen, and bile were negative for pathogenic organisms.

The diagnosis in this case was based entirely on a comparison of the histopathologic observations with those in known cases of infectious mononucleosis, the appearances in the spleen and lymph nodes being especially clear-cut.

Case 4 (A I P No 100124) The patient, a white officer 29 years old, had felt achy, tired, and listless for 11 days, during the last 3 of which were added the symptoms of fever, aching of eyes, anorexia, epigastric distress, abdominal cramps, and diarrhea. On the tenth day cramps and diarrhea ceased, and severe frontal headache and nasal discharge began. On admission to the Station Hospital, Fort Benning, on October 11, 1943, it was noted that his skin was flushed and dry, his pharynx reddened, and his sclerae slightly icteric. Cervical lymph nodes were enlarged, the epigastrium tender, and the spleen palpable.

Laboratory Data

	R B C (M)	Hb (%)	W B C (Thou sands)	Neutro (%)	Lympho (%)	Mono (%)	Eos (%)	Hetero- phil Agglutination	Cephalin Flocculation Test
Oct. 12	3.9	80	17.5	8	72	20		1:1280	4+
Oct. 13	4.1	90	12.5	74	25	1			1+
Oct. 19	3.5	75	13.2	50	39	11			
Oct. 23	2.9	65	14.0	84	8		8		
Oct. 25	3.8	80	10.7	73	21		6	1:160	

Urine icteric index, and prothrombin time were within normal limits and the Kahn test was negative. The patient had hookworm disease, which probably accounted for the slight eosinophilia.

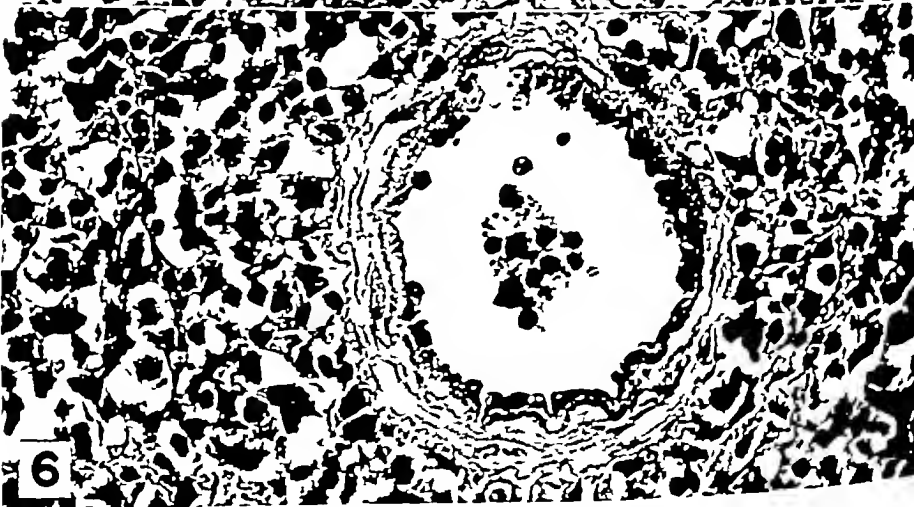
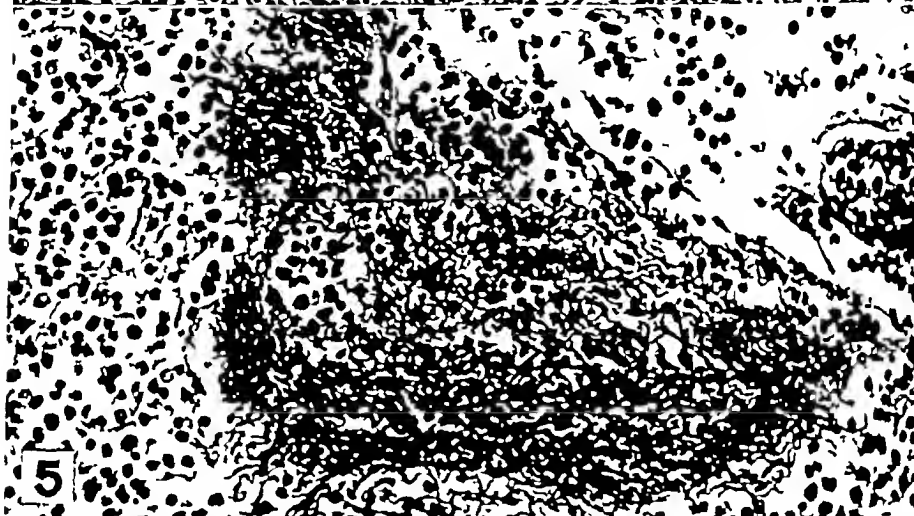
An attempt was made to palpate the spleen on October 18 (the eighteenth day of the disease) whereupon symptoms indicative of splenic rupture suddenly appeared and were confirmed at operation 5 hours later. The spleen was not weighed but it measured 19 x 11 x 2.5 cm. Its capsule was thin, its pulp semifluid, and a huge rent extended from the upper pole into the midzone in a ragged, serrated contour. The reasons for the diagnosis in this case are quite clear. The patient left the hospital on November 10, 1943, in good condition.

Case 5 (A I P No 144648) A 19 year old white soldier was admitted to the Station Hospital, Kearns Army Air Base, on August 17, 1943, complaining of recurrent severe headaches which awakened him at night, he was nauseated and dyspneic. His temperature was 103° F, pulse 106 and respirations 24.

FIG. 1. Minimal changes in the capsule of the spleen comprise a loosening of the structure by edema and an increase in nuclei per unit area, most of which are those of atypical lymphocytes. A subcapsular zone of edema is also evident. X 280.

FIG. 2. Moderately advanced changes in the capsule of the spleen are shown. Both edema and cellular infiltrate are clearly defined. X 500.

FIG. 3. The capsular lesion here is so marked that the original structure can hardly be identified. The capsule appears stretched and ready to rupture although the section was taken some distance from the actual break. X 500.



per minute Tonsils and pharynx were inflamed, the lymph nodes generally enlarged, and the spleen palpable

Laboratory Data

	R.B.C (M)	Hb (Gm)	W.B.C (1000's)	Neutro (%)	Lympho (%)	Mono (%)	Hetero- phil Agglutination	Platel (1000's)
Aug 17	4 5	14	10 6	71	29	0		
Aug 23	4 7		9 2	69	31			
Aug 23	4 3	13 5	24 8					
Aug 23	3 2	12	21 1	30	70		1 40	
Aug 24	3 0	10	18 3	32	68			
Aug 25							1 112	
Aug 27	4 45	11 5	14 8	74	26			
Aug 28	4 95	13	16 4	70	30			
Aug 31	4 50	15	14 0	75	25			
Sept 4	4 52	15	21 8	80	20			
Sept 4			18 9					271
Sept 13	4 8	13	14 0	76	24			
Sept 16	4 52	14	15 0	62	38			
Sept 18	4 73	14	17 0	80	20			
Sept 20	4 00	13	14 8	70	30			
Sept 23	4 43	14	10 0	70	30			
Sept 27	4 60	13 5	6 5					
Sept 30	4 6	13 5	9 5	69	31			
Oct 4	4 64	13	9 0	69	31			
Oct 7	4 80	14	9 3	54	46			
Oct 11	4 95		7 8					

A blood culture, agglutination tests, and Kahn reaction were negative

On October 23 while returning from the latrine the patient complained of sudden pain in the left upper quadrant of the abdomen and went into shock. The diagnosis of ruptured spleen was made. Splenectomy was performed and clotted blood was removed from the peritoneal cavity. The spleen weighed 425 Gm and measured 16 x 12 x 7 cm. On the diaphragmatic surface was a huge gaping capsular rupture running the entire length of the organ and measuring 8 cm across. The postoperative course was marked by pneumonia which was readily controlled by sulfonamide therapy, otherwise the patient made a good recovery.

The diagnosis in this instance rested on the clinical course—lymphadenopathy, and splenomegaly, fleeting but significant lymphocytosis, and a heterophil antibody titer which rose from 1:40 to 1:112.

Case 6 (A.I.P. No. 94425). A 26 year old white soldier had had a cold with a slightly productive cough for a month prior to his admission to the Station Hospital, Camp Butler, on February 14, 1943. Twelve hours previously while getting into bed, he had experienced severe upper abdominal pain followed by nausea and vomiting. In an hour, the pain had lessened but it gradually localized in the left upper quadrant and was exaggerated by breathing. There was generalized direct and rebound abdominal tenderness most marked in the right lower quadrant but no other physical findings of significance. Laparotomy 4 hours later (16 hours after acute onset) disclosed intraperitoneal hemorrhage and rupture of the spleen which was 4 times normal size.

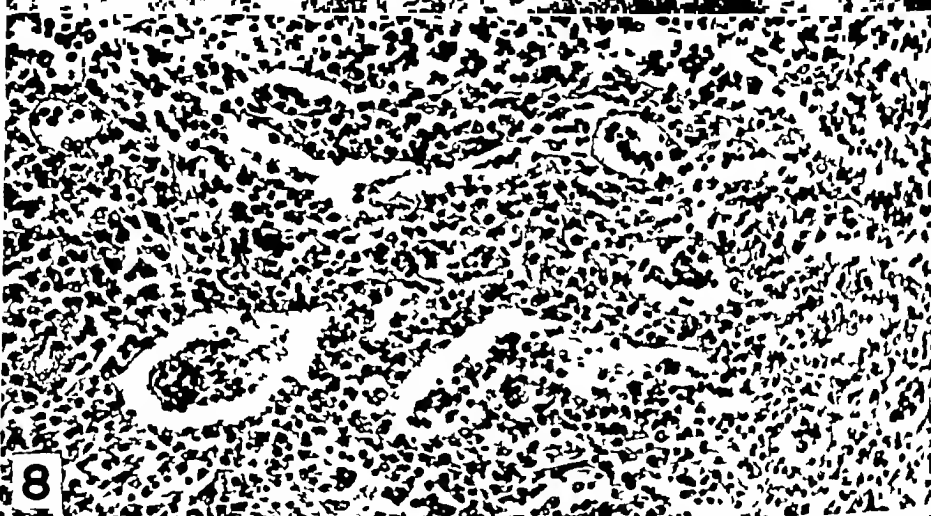
FIG 4 Moderate infiltration of a trabecula by normal and abnormal lymphocytes $\times 200$

FIG 5 A reticulin stain (Wilder) of the trabecula shown in Fig. 4 brings out a loosening of the framework $\times 330$

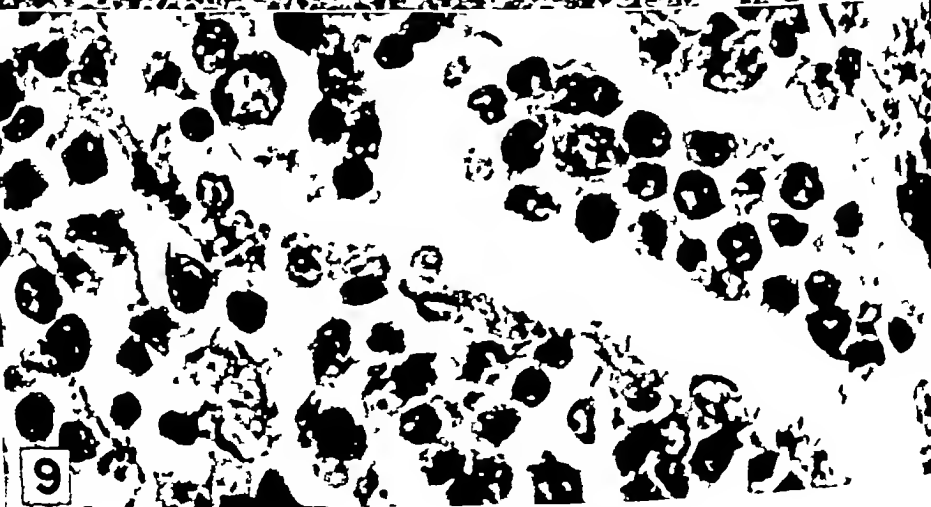
FIG 6 An intratrabeular artery marks the previous site of a trabecula of which virtually no other vestige remains $\times 500$



7



8



9

	Laboratory Data							Platelets (1000 s)	Retic (%)
	R B C (M)	Hb (%)	W B C (1000 s)	Neutro (%)	Lympho (%)	Mono (%)			
Feb 14			14 8	47	41	11			
Feb 15	4 1	85	22 9	44	53	3			
Feb 16	3 03	68	20 8						1 8
Feb 17	3 75	90	16 3	55	44	1			
Feb 18	2 46	64	12 7	53	43	4			
Feb 19	2 54	70	18 8	50	46	4			
Feb 20	3 73	68	12 7	49	44	7			8 3
Feb 21	3 15	85		55	27	11			2 0
Feb 23	3 05	68	14 1	69	30	1			
Feb 24	3 25	70	19 7					116	5 4
Feb 24	3 57	73	26 8					118	5 8
Feb 27	3 53	70	9 6	81	14	4			
Mar 1	4 31	90	8 2	70	30				
Mar 3	4 05	85	10 1	72	21	4			
Mar 6	4 10	85	15 7	79	21			150	2 0
Mar 8	3 92	85	11 5	68	30	2			
Mar 14	3 86	80	11 9	66	34				
Mar 16	3 75	78	9 5	58	39	3			

Heterophil antibody titration was not performed. Other laboratory data were essentially normal.

On the ninth postoperative day the patient suffered a minor pulmonary embolism and on the seventeenth day there was clinical evidence of thrombophlebitis of the deep veins of the left leg. A more severe embolism occurred on the twenty ninth day and he died two days thereafter.

This case is classified as infectious mononucleosis because of the history of a cold lasting for a month, a numerical increase in lymphocytes, some of which were abnormal, and the histologic appearances of the enlarged spleen and other tissues which were identical with those in authenticated cases of the disease.

Case 7 (A I P No 67260). A 21 year old white man entered a civilian hospital on February 3, 1940 complaining of bleeding from the nose and gums. Bruises were seen on his legs, and he had passed dark-colored urine. One week earlier the patient had had an attack of flu from which he had virtually recovered. Physical examination disclosed bleeding from the nasal and oral mucosae, ecchymoses of the skin, and an enlarged spleen.

	Laboratory Data							Platel (1000 s)	Bl T (min)	Clot Retraction
	R B C (M)	Hb (Gm)	W B C (1000 s)	Neutro (%)	Lympho (%)	Mono (%)	Blast (%)			
Feb 3	4 7	14	10 7	21	73	6			23+	
Feb 4								18 8		
Feb 6	4 1	11	11 0	15	75*	8	2			
Feb 8	3 6	12	9 3	33	61	4				
Feb 9	3 6	12	9 0	35	63	2				0

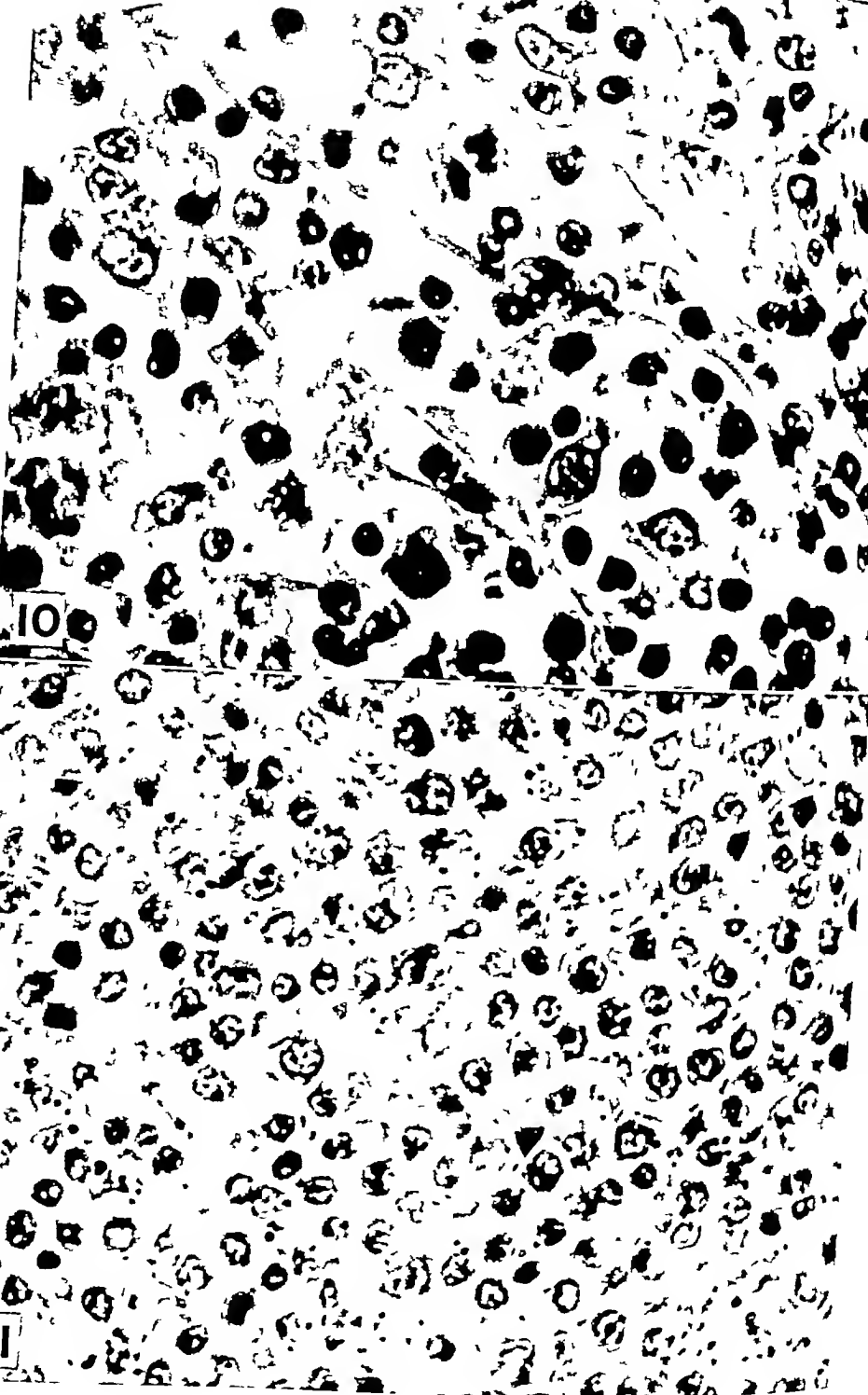
* 45 per cent noted as young cells.

A working diagnosis of thrombocytopenic purpura was made and the patient was given increasing doses of moccasin venom and small blood transfusions. His condition changed but little until February

FIG 7. The general pattern of the spleen is obscured by cellular proliferation in the pulp; the follicles being small, indistinct, and widely spaced, and the sinuses are barely discernible. $\times 35$.

FIG 8. A higher magnification in the same case shows the lumina of most of the sinuses occupied by clumps of normal and atypical lymphocytes. $\times 200$.

FIG 9. Still higher magnification shows details of the cells in both sinus and pulp; a few plasmocytes can be made out in the pulp. $\times 1000$.



10 (about the fourteenth day of illness) when signs of intraperitoneal hemorrhage became evident and he died

The peritoneal cavity contained 2.5 liters of fluid blood in addition to large clots. The spleen, which was not weighed, had a large tear through the capsule into the pulp for a few millimeters, most of the capsule was separated from the soft, flabby, maroon-colored parenchyma by an interposed layer of clotted blood.

Despite the lack of heterophil antibody titration, we regard this as a case of infectious mononucleosis in which the clinical manifestations were chiefly those of thrombocytopenic purpura, a relatively rare form of the disease but one which already has been described.^{5*} The prodromal symptoms resembling influenza and the absolute lymphocytosis with abnormal cell forms in the peripheral blood tend to substantiate this assumption. Finally the histopathologic changes in the tissues, especially in the lymph nodes and spleen, were characteristic of infectious mononucleosis.

DISCUSSION

Reasons for the diagnosis of infectious mononucleosis in these 7 cases have been stated at the end of each case report. The clinical data in 4 are deficient, chiefly because the disease was not suspected in 3 and death was so sudden in the fourth. The information available in all but case 3 is, however, entirely consistent with the diagnosis of infectious mononucleosis. Histopathologic findings in the ruptured spleens were singularly constant in all 7 cases and were, furthermore, identical with those in intact spleens from patients with infectious mononucleosis who had died from other causes. The characteristic picture was so uniform that sections of spleen from infectious mononucleosis shuffled with those from a wide variety of diseases could readily be distinguished and grouped together again. Consequently a single composite description will suffice for the entire group.

Histopathology of the Spleen. At the Army Institute of Pathology there are 44 cases listed as spontaneous rupture of the spleen with material available for microscopic study in 33, 11 cases of recurrent malaria exist merely as records. Our analysis of the 33 cases established certain criteria for the objective microscopic diagnosis of infectious mononucleosis (table 1), some of these features have not been mentioned in the descriptions of earlier authors.

In the capsule we noted an infiltration by lymphocytes and large mononuclear cells (to be described later) in all but 2 cases, had more sections been available in the latter instances, we suspect that these spleens would not have been exceptions. Ziegler⁷ also observed a moderate cellular invasion of the capsule, but Darley et al.⁸ made the direct statement that the capsule and trabeculae of the spleen appeared normal except where they had been distorted by hemorrhage into the adjoining substance. Minimal capsular changes, which may be regarded by some as of no consequence but which we believe to be significant, are shown in figure 1. There is edema of the capsule and the immediately subjacent pulp, and an increase in nuclei per unit area over the normal. Figure 2 at higher magnification presents

FIG. 10. The pulp of the spleen in some cases is loosely arranged. The admixture of normal and abnormal lymphocytes, reticulum cells, and plasmocytes is clearly seen. $\times 1000$.

FIG. 11. The splenic pulp may be densely packed with cells, most of which are atypical lymphocytes. The deposit of formalin precipitate was intentionally left in this section to illustrate the necessity of differentiating it from malarial pigment. With polarized light formalin precipitate is doubly refractile, whereas malarial pigment is not. $\times 1000$.



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more marked edema and round cell reaction, while the capsule seen in figure 3 is so cellular as to have virtually lost its identity, and appears stretched and ready to rupture. None of the sections illustrated were taken near the areas of actual rupture of the several spleens.

The *trabeculae* are infiltrated by mononuclear cells to an even greater degree than the capsule, frequently undergoing complete dissolution. The trabecula in figure 4 displays moderate change, and a reticulum stain (fig 5) discloses loosening and fraying of the reticular fibrils. In figure 6 the process is so advanced that the site of the trabecula is identified only by the remaining small intratrabecular artery.

The *splenic pulp* is increased in bulk and the architectural pattern is blurred, principally because of excessively cellular cords of Billroth which compress the sinuses and render them relatively bloodless (fig 7). The blood sinuses contain clumps of mononuclear cells (figs 8 and 9) which frequently fill the spaces and together with the swollen sinus endothelium serve to obscure the pattern still further. Cells may be rather loosely dispersed (fig 10) but are more frequently closely packed (fig 11), sometimes to a degree simulating leukemia. *Follicles* are few per unit area, owing to the increased amount of intervening pulp (fig 7), they are generally small, for the most part lack germinal centers, and are composed of mature lymphocytes. The follicular margins are poorly defined and the progressive hyperplasia of the pulp tends to obliterate some of the follicles completely.

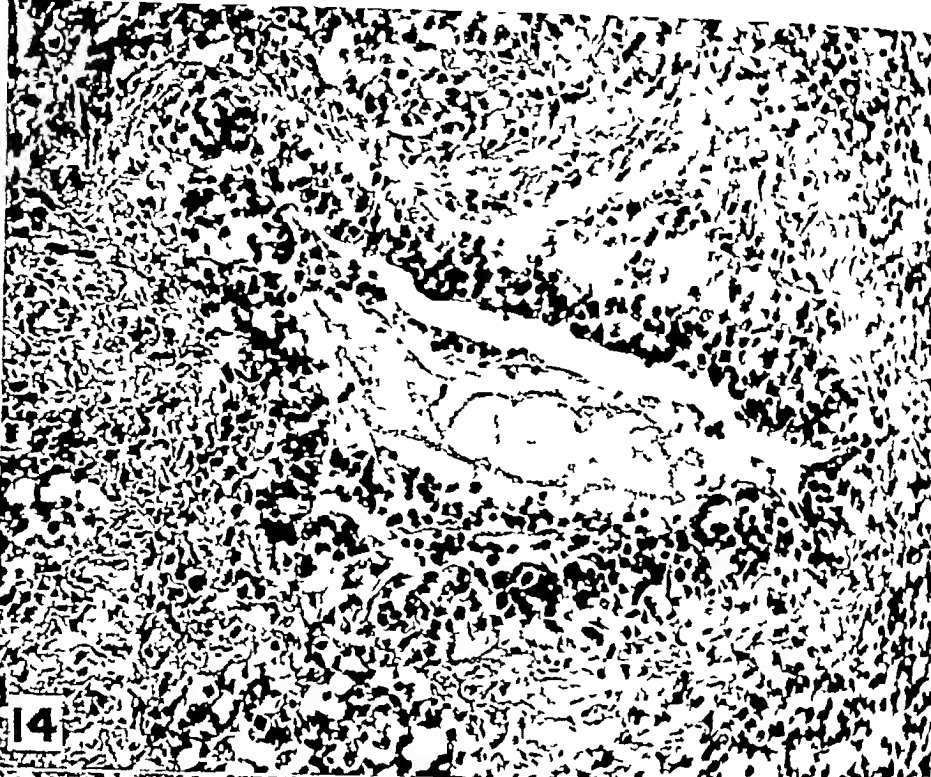
The *predominant mononuclear cell* of which we have spoken, when stained lightly with hematoxylin and eosin in thin sections, varies from 12 to 15 microns in diameter, occasionally larger, and is round except when distorted by crowding (figs 9, 10, and 11). The cytoplasm is homogeneous and faintly acidophilic. The centrally or eccentrically placed nucleus is sharply delineated by a thin membrane which blends with the marginal chromatin particles, chromatin is irregularly distributed to lend a mottled appearance, and it occasionally forms angulated bars. Indentation and folding of the nuclei can be demonstrated in relatively few cells. It is virtually impossible to determine in the sectioned material whether a true nucleolus is present or not, nor could fenestration be evaluated. We regard these cells as atypical or abnormal lymphocytes, closely related to, if not identical with, those found in the peripheral blood. The variation in descriptions of morphologic details of the cells among various authors may be explained in part on differences in the technical methods employed. Furthermore, word-pictures have shortcomings which can be overcome only by direct observation of well prepared sections, after a little experience the recognition of these cells is not difficult.

Other cellular components of the splenic pulp comprise lymphocytes, reticulum cells, and plasmocytes in varying proportions. In case 7, in which there was moderate anemia, the spleen was the seat of extramedullary hematopoiesis.

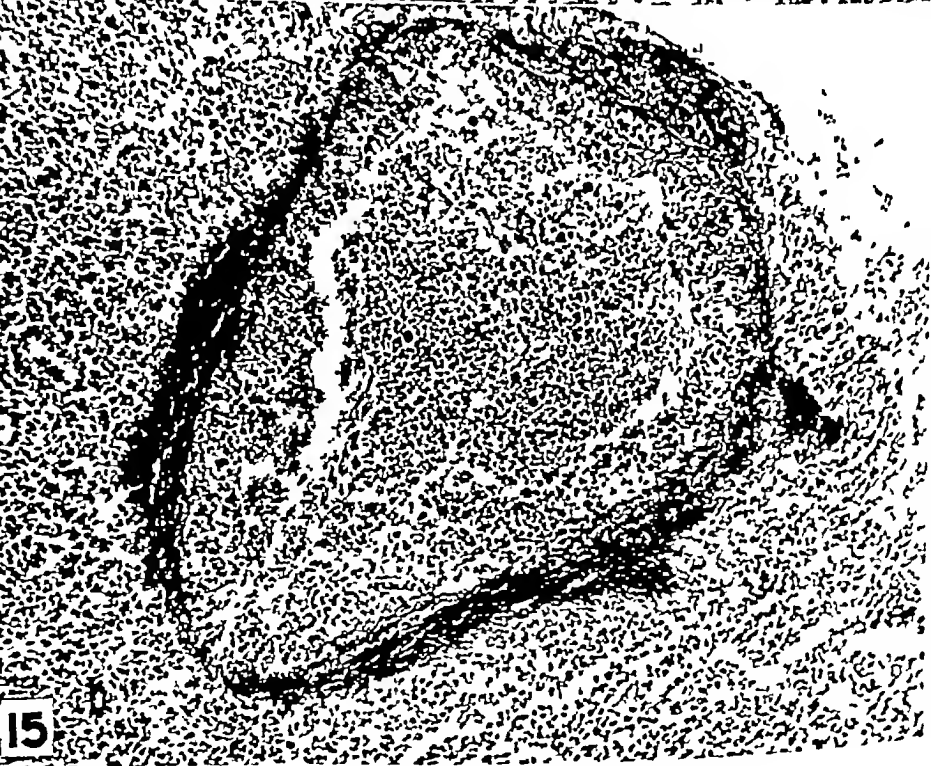
A striking histologic change is consistently observed in the *blood vessels* of the spleen. Small arteries are ensheathed by normal and abnormal lymphocytes which

Fig 12. A trabecular artery with slight subintimal and extensive adventitial aggregations of mononuclear cells, most of which are atypical lymphocytes. $\times 200$

Fig 13. The same vessel stained by Wilder's method for reticulin. $\times 200$



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completely separate them from the surrounding trabecular substance (figs 12 and 13) The subintimal zone of intratrabecular veins displays a similar cellular reaction (figs 14 and 15) These vascular lesions are frequently found in leukemia⁸ and scarlet fever,⁹ occasionally in chronic malaria and in acute fulminating infections The subintimal reaction in the veins is also common to allergic states of various types,¹⁰ especially drug sensitivity,¹¹ in which the patient survives 24 hours or longer It is thought by some observers that this layer of cells is merely an extension from the hyperplastic pulp, but it has been seen in veins of other organs We did not find either arterial or venous lesions in the hyperplastic spleens of typhoid fever, diphtheria, measles, peritonitis, subacute bacterial endocarditis, plague, anthrax, military tuberculosis, influenza, lobar pneumonia, periarteritis nodosa, polycythemia vera, thrombocytopenic purpura, and familial hemolytic jaundice

Regarding the nature of the cellular reaction in the capsule, trabeculae, and vessels, we are in accord with Jaffe's⁸ view that this is a metaplastic process, the cells stemming from fixed tissue elements, rather than a direct infiltration through their own motility

Relation of Splenic Changes to Rupture of the Organ It is obvious from these histologic descriptions that *the basic structure of the spleen is weakened in infectious mononucleosis* The dilution and even dissolution of the capsule and trabeculae illustrated in figures 1 through 6, the result of a cellular reaction of varying intensity within these structures, are perhaps the most important factors Small wonder then that these rapidly expanding organs with damaged envelopes and framework occasionally rupture Rather it is surprising that they do not rupture more often The changes in this group of cases are not fortuitous, as we observed them consistently in the intact spleens of patients who died from some other cause

The recorded data on the size of the spleen indicated an enlargement to 3 or 4 times normal, this held true for our series and the other 3 reported cases Table 1 supplies evidence that normal-sized spleens are not apt to rupture spontaneously Rapidity of enlargement, as from the acute hyperplasia in infectious mononucleosis, with consequent stretching of the capsule and thinning of trabeculae, constitutes an obvious factor This is also emphasized by the spleens in our group of chronic malaria, in which rupture invariably was associated with a recurrence when active hyperplasia of the splenic pulp took place

The time element proved interesting The spleen in infectious mononucleosis is not likely to rupture during the first 2 weeks of the disease, the estimated interval between onset of symptoms and the accident in our cases 1, 2, 4, 6, and 7 was 21, 15, 18, 30, and 14 days respectively In the other cases reported the spleens ruptured on the seventeenth, eighteenth, and twenty-fifth days This suggests that not until the third week have the capsular and trabecular changes progressed sufficiently to permit rupture, either spontaneous or the result of slight trauma

FIG 14 Subintimal layer of mononuclear cells in a trabecular vein $\times 300$

FIG 15 A large vein with a thick inner lining of mononuclear cells, readily distinguishable from the mass of cells which fills the lumen and which probably was squeezed in from the pulp during manipulation of the organ Wilder reticulin stain $\times 130$

Relation of Trauma to Rupture of the Spleen Division of splenic rupture into categories of spontaneous and traumatic is not entirely accurate. There are unquestionably cases catalogued as spontaneous in which the diseased spleen would not have ruptured but for some trivial injury, unnoticed or forgotten. It is certain that in some of these instances the actual rupture was antedated several days by a subcapsular hematoma evidenced by a layer of partially organized blood beneath the capsule. That the bleeding may be intermittent is suggested by bouts of epigastric distress, nausea, vomiting, and diarrhea which have been observed at intervals preceding the time of actual rupture, in such cases lamellae of clot represent the successive bleeding episodes. One case in our series presented a definite history of rupture having been induced by palpation of the spleen during physical examination, in another, rupture occurred while the patient was getting into bed, and probably was due to a slight jar or twist. In still a third the symptoms of splenic rupture began while the patient was returning from the lair. Whether increased portal pressure incident to straining at stool was a factor can not be said with certainty. Similar exciting causes may precipitate rupture of the spleen complicating conditions other than infectious mononucleosis.

Although rupture of the spleen is apparently a very infrequent complication of infectious mononucleosis, it is well for the clinician to bear this possibility in mind. We recommend that, when infectious mononucleosis is suspected, enlargement of the spleen be taken for granted, palpation be omitted, and the diagnosis established by the more certain means available.

SUMMARY AND CONCLUSIONS

1. Seven cases of ruptured spleen as a complication of infectious mononucleosis are described and reference made to the 3 cases previously recorded in the literature.

2. It proved possible to make an objective histologic diagnosis of infectious mononucleosis from well prepared sections of the spleens. The diagnosis was based on (a) a blurred architectural pattern due chiefly to large numbers of atypical lymphocytes diffused throughout the pulp and clumped in the blood sinuses, (b) small, poorly defined follicles, usually without germinal centers, in less than usual numbers per unit area, (c) cellular infiltrates, composed largely of normal and atypical lymphocytes, in the capsule and trabeculae, in the adventitia of small intratrabecular arteries, and in the subintimal zone of collecting venous sinuses and intratrabecular veins, (d) swelling of the lining or attached cells of the blood sinuses.

3. Infiltration of the capsule and trabeculae reached considerable proportions, occasionally to the point of complete dissolution of these structures, and served as a predisposing cause of rupture. The same changes were noted in intact spleens from fatal cases of infectious mononucleosis.

4. The spleen in infectious mononucleosis was 3 to 4 times normal size and ruptured during the third or fourth week of the disease.

5 The importance of trivial injury as the exciting cause of so-called spontaneous rupture of the spleen has been emphasized. It is recommended that extreme caution be employed during attempted palpation of the spleen in a suspected case of the disease. When the diagnosis is obvious, splenic palpation may well be omitted.

NOTE: Since preparing this article we found that 2 of our cases recorded above were included in a report by Littlefield (Surg., Gynec. & Obst. 82: 207, 1946) dealing mainly with clinical and surgical aspects of spontaneous rupture of the spleen *per se*, without particular regard to diagnosis of the underlying disease. We noted, however, that in his case 1 (our case 5) the statement is made that infectious mononucleosis was suspected and ruled out and the diagnosis of ruptured acutely hyperplastic spleen offered. Our reasons for the diagnosis of infectious mononucleosis in this case are presented in the text of our paper and the splenic pulp is pictured in figure 11. In Littlefield's case 3 (our case 1) he quotes from the clinical records, impression: reticulum cell sarcoma of spleen. We direct attention to our figures 7 through 10 from this case which we regard as particularly illustrative of the typical changes in the spleen of infectious mononucleosis.

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INFECTIOUS MONONUCLEOSIS COMPLICATED BY SPONTANEOUS RUPTURE OF THE SPLEEN AND CENTRAL NERVOUS SYSTEM INVOLVEMENT

By STUART L. VAUGHAN, M D, J. SUTTON REGAN, M D,
AND KORNEL TERPLAN, M D

WHILE infectious mononucleosis is usually a benign disease, serious complications develop occasionally. Spontaneous rupture of the spleen has been described in four well documented reports,¹⁻⁴ and two other cases^{5,6} in which the evidence is not conclusive have been cited. Central nervous system involvement has been described also, and the literature concerning this complication has been reviewed recently.⁷⁻⁹

CASE REPORT

A married white male, aged 38, was admitted to the hospital November 3, 1944. About one week previously he had complained of stiffness in the back of the neck. This symptom continued steadily for one week before admission became exaggerated. At this time he developed an intractable headache which was chiefly frontal in distribution and felt feverish. Treatment at home brought no relief and he was admitted to the eye, ear, nose and throat service for observation. After admission he noticed some blurring of vision for the first time.

Past history revealed that the patient had been subject to frequent colds and sore throat. He had undergone appendectomy in 1919, tonsillectomy in 1929 and hemorrhoidectomy in 1940. Otherwise his health had been good.

Physical examination on admission revealed that he did not have the appearance of serious illness although he was complaining of pains in the head and throat. The temperature was 100.0, the pulse rate 102 and the respiratory rate 24. The conjunctivae were inflamed. The throat was red and inflamed. Tonsils were missing. The lymph nodes were not enlarged and no abnormal signs were found on examination of the heart, lungs or abdomen. The appendectomy scar was noted. Reflexes were hyperactive. A yellowish maculo-papular circinate lesion the size of a ten cent piece was found on the inner surface of the right thigh near the groin.

Routine blood counts showed the following: R B C 5,100,000 per cu mm, Hb 14.4 Gm per 100 cc, W B C 13,000 per cu mm. Band form polymorphonuclears, 25 per cent; filaments 3 per cent; eosinophils 1 per cent; basophils 1 per cent; lymphocytes, 66 per cent; monocytes 4 per cent.

The laboratory reported that many of the lymphocytes were large and deeply basophilic and characteristic of types I and II leukocytoid lymphocytes as described by Downey.¹⁰

X-rays of the paranasal sinuses were negative.

No changes occurred in the clinical condition until the third day after admission (November 5th). At noon the patient went to the lavatory feeling fairly well. He sat down to defecate but does not know whether he had a movement or not. He stated that there was no undue straining. Then he seemed to lose consciousness momentarily and came to with his head lying against the tank. He arose, went to the nurses' station and then to bed. He felt weak but otherwise did not feel badly. A short time later his lunch was brought in. He ate only a little ice cream and felt very weak. Because of weakness and marked perspiration, the nurse called a physician. The pulse rate was 140 and the blood pressure was 60 systolic, 30 diastolic. The abdomen was tender throughout, but most markedly so in the umbilical region. It was not until several hours later that pain developed. Definite spasticity was noted but this was brief in means boardlike. The red count was 4,000,000 and the hemoglobin 10 grams.

One of us (J. S. R.) was called in surgical consultation during the evening. Spasm and rigidity were marked in the upper abdomen and less so in the lower abdomen. Under observation the rigidity became more marked. Faint peristalsis was present.

Treatment for shock, including transfusion of blood was instituted, and twelve hours after the onset laparotomy was performed. At operation the peritoneal cavity showed evidence of massive hemorrhage. The spleen was found enlarged to about four times its normal size and very soft. A large amount of clotted blood was found adherent to the lateral and diaphragmatic surfaces, and from beneath the clots fluid blood was observed to escape freely. With some difficulty, because of the softness and friability of the organ the spleen was removed and the wound closed.

PATHOLOGIC-ANATOMIC REPORT

3287 1944 *Gross Report* The spleen is moderately enlarged, weight 400 grams, measurements $18 \times 9.5 \times 3.5$ cm. Within the diaphragmatic surface near the upper pole and close to the anterior margin there is a small irregular tear nearly 2 cm. in length. It is partially covered with clotted blood and appears to be about 1 cm. deep. On the cut surface the consistency of the spleen is considerably decreased. The markings are completely obscured, the malpighian follicles are not visible and the entire splenic substance is in a state of uniform swelling and of grayish pink color. About the tear there are distinct hemorrhages within the pulp. Small localized hemorrhages from 1 to 4 mm. in diameter are seen in other parts of the spleen also, especially close to the capsule. The larger ones, including those about the tear, have an infarct-like appearance. (This latter feature grossly suggested the possibility of small embolic lesions as seen in bacterial endocarditis.)

Microscopic Report (Fixatives used: Zenker, Orth, Formaldehyde) In the low power magnification the structure of the spleen appears to be distinctly changed. While in some areas the malpighian follicles are still noticeable, in others they seem to blend with the hyperplastic red pulp. In the areas with hemorrhages they stand out more prominently. The trabecular system is indistinct, only the trabeculae containing larger blood vessels being clearly seen. So-called germinal centers cannot be recognized within the malpighian corpuscles.

With higher magnification the most outstanding feature is that the sinusoids are obscured, only close to the hemorrhagic areas a few of them can be recognized. The bulk of the cells composing the swollen pulp seems to stem from the reticulum of the spleen, gradually blending with the malpighian follicles. There is, apart from the hemorrhagic zones, but little blood between the reticulum meshes and in those sinusoids which still can be recognized. The following cells are most prominent: lymphoid elements with round nuclei and distinct though narrow rims of cytoplasm, slightly larger cells with round or oblong nuclei with indentations within the nuclear membrane and very distinct cytoplasm. These same cells frequently appear as the sinus endothelial cells. Leukocytes are comparatively few in number. Likewise only a few eosinophilic leukocytes and rare plasma cells are seen. Macrophages free in the sinusoids, are also comparatively infrequent. Phagocytosis of erythrocytes is by no means prominent. The most outstanding feature is the rather regular cytoplasm in all the cells making up the bulk of this peculiar cellular hyperplasia.

There is a very rare follicle presenting enlargement of reticulum cells as seen in the germinal centers. However, there is a very marked degree of nuclear disintegration with many small chromatin particles within these cells, many of which have dark eosinophilic cytoplasm and blurred cellular borders. The arteries within the follicles show irregular hyaline degeneration in their walls. Some nuclear disintegration is also seen within the splenic trabeculae. The proliferation of the reticulum cells finally seems to extend through the trabeculae in some areas and to encroach upon the endothelial lining of the splenic veins. This is seen also within the walls of some arteries in the trabeculae. Sections stained for iron fail to show any granular hemosiderosis or diffuse imbibition.

In sections stained with methylene blue the larger lymphoid cells clearly show disintegration of nuclear substance into small fragments. Some of these fragments resemble bacterial inclusions of diplococcus form, while others are less homogenous and of various sizes. Gram stains give an entirely negative result. The bacteria-like structures seen in thin sections stained with methylene blue had to be interpreted as nuclear debris. They were seen also in some endothelial cells within packed sinusoids.

In comparing the histological picture with those reported by Warren,¹ Ziegler,² and Black,³ it is clear that our findings are very similar to theirs. In all of these reports it is stated that the sinusoids are less prominent than normal. The indistinct follicular architecture and also the absence of germinal centers are stressed by Ziegler and Black in particular. The large lymphocytes and reticulum cells mentioned by Ziegler and the larger lymphoid cells with characteristic nuclei and abundant cytoplasm in the

report of Black are apparently identical with those described in our report as small and dark cells which seemed to be in connection with the reticulum of the pulp and appearing frequently in the endothelial cells wherever packed smears could be made out.

Sinusoids nearly filled with young lymphocytes, monocytes, and an occasional polymorphonuclear cell also as the characteristic histological feature in the case of Davis, MacFee, Wright and Allen.¹

When we analyzed the histological picture we were impressed by the comparative absence of polymorphous leukocytes and large endothelial cells with active erythrophagocytosis as seen frequently in the swelling of the spleen reactive to various bacterial infections (meningococcus septicemia, streptococcus acute bacterial endocarditis). The absence of larger numbers of leukocytes in particular was emphasized as the soft consistency noticed in the gross specimen had directed our expectation toward a septicemic reaction.

The picture then that we found, is fully compatible with the hematological and serological data of infectious mononucleosis. Whether it is in itself sufficiently characteristic for the morphological diagnosis of this disease cannot be stated with any degree of certainty at the present time.

The immediate post-operative course was attended by moderate fever for eleven days. Salicylate was administered until the third day, when it was discontinued because of the appearance of a

TABLE I—Blood Changes

	1944											1945
	11/3	11/5	11/7	11/9	11/13	11/20	11/22	11/26	11/27	11/28	11/29	12/11
Erythrocytes (million)	5.10	3.96	3.80	3.40		4.20					4.6	4.5
Hemoglobin (grams)	14.4	10.0	11.2	9.6		12.2					13.1	13.1
Leukocytes	13,000		17,000	26,000		14,000	18,000	17,000			20,000	13,000
Myelocytes (%)				1								
Young forms			1									
Band forms	25	Splenectomy	30	18		27	14	21	36	47	74	1
Filament forms	3		25	14		15	50	44	33	27	42	4
Eosinophils	1			2						1	3	2
Basophils	1			2		2			1	1		1
Lymphocytes	66		41	47		44	33	24	25	1	71	31
Monocytes	4		2	8		2	3	11	5	10		12
Normoblasts			1*	8								
Heterophile titer					1280	320					160	1

* Percentage of nucleated cells

erythematous eruption. On this day there was a chill. The urinary sediment was found to be normal with red and white cells.

The patient complained of diplopia. Ophthalmological examination by Dr. Ivan Koetz showed paralysis of the left external rectus muscle. Both disks were fairly well defined but the veins were slightly engorged. No definite edema, hemorrhages or exudate were found. Pronounced bilateral ankle clonus was present.

The heterophile agglutination test done on November 13th was positive in a dilution of 1:128. On November 15th a small incisional abscess was drained. Bacteriological examination of the exudate revealed anaerobic streptococci and small anaerobic Gram positive gas-producing bacilli. Penicillin was given for two days and the temperature returned to normal promptly. A test for cold agglutinin on November 17th was negative.

On November 18th a clinical examination from the hematological standpoint was made by Dr. (S. V.) (This examiner had confirmed the diagnosis of infectious mononucleosis from the laboratory standpoint previously.) The skin color was normal. No hemorrhagic signs were present. The cervical, axillary, epithrochlear, and inguinal lymph nodes were not enlarged. The only node palpable was a small one in the left side of the neck. Ocular movements were practically normal and diplopia had disappeared. However, all tendon reflexes were greatly exaggerated. Ankle clonus was easily elicited bilaterally and was not sustained. Patellar clonus was absent. Plantar reflexes were normal and no other abnormal neurological findings were noted.

Lumbar puncture done November 20th showed initial pressure of 175 mm. of water. WBC 100.

compression the pressure rose to 220 mm within 10 seconds and returned to 175 mm within 10 seconds of releasing the compression. Respiratory and arterial pulsations were present. The fluid was clear and colorless. The cell count was 8 white blood cells per cu mm. The Pandy test and nitric acid ring test were slightly positive. Copper reduction was prompt. The colloidal gold curve was 1111000000. Complement fixation tests were negative and cultures were sterile. Glucose was 131 mg per cent and protein was 0.116 Gm per cent.

On November 27th, after several days of progressive improvement, the patient complained of dull steady pain in the right flank accentuated by deep respiration. Temperature rose to 103.2, the pulse rate to 132, and respiratory rate to 40, otherwise physical signs were indefinite, and radiographic examination revealed no abnormality. However, within two days signs of fluid in the right pleural cavity were present, radiographic examination was confirmatory. On December 4th the fluid removed from the chest was faintly turbid. It contained 31,500 R B C and 3,300 W B C per cu mm. Stained films of sediment showed no bacteria and cultures including special ones for tubercle bacilli, were sterile. Cytological examination showed the following: young forms of polymorphs, 2 per cent, bands, 7 per cent, filaments, 3 per cent, lymphocytes, 20 per cent, monocytes, 5 per cent, plasma cells with highly vacuolated cytoplasm, 22 per cent, mesothelial cells, 41 per cent.

The febrile reaction subsided within a few days, and the patient was discharged from the hospital on December 16th, 44 days after admission. At that time he was in good condition, and all abnormal signs had disappeared.

On February 23rd, June 5th, and November 14th, 1945, he was re-examined by one of us (S V). On each occasion he appeared to be in excellent health and had no complaints except that he tired more easily than before his illness. Lymph nodes were barely palpable in the neck and axilla. No abnormal ocular or neurological signs were present. The blood findings throughout the illness are shown in Table 1.

DISCUSSION

The clinical course in this patient was not typical of that seen in the majority of cases of infectious mononucleosis. Peripheral lymph node enlargement and ulcerative manifestations were not present at any time. Even after the diagnosis was known a careful examination failed to disclose these signs. The first evidence of the disease was disclosed by the laboratory technician in the routine blood count. However, before this evidence was evaluated and re-checked the acute crisis of splenic rupture had taken place.

The significance of the blood findings was not appreciated by the physicians in attendance up to this time and infectious mononucleosis was not considered in the preoperative differential diagnosis. In fact, it must be acknowledged that the signs did not seem to indicate splenic rupture, and this finding was disclosed only during the operation after an initial upper right rectus incision had been made.

Examination of the spleen in the tissue laboratory presented similar difficulties. The initial findings suggested an acute inflammatory process and a painstaking search for bacteria was instituted. It was only after this search failed to reveal any organisms and after the diagnosis of infectious mononucleosis became apparent from other evidence that the findings in the spleen were regarded as compatible with this diagnosis.

These difficulties in preoperative and pathological diagnosis are similar to those described by other authors. Preoperative diagnosis will only be improved by wider dissemination of the knowledge that spontaneous splenic rupture due to infectious mononucleosis is a possibility in surgical crises. As to the tissue diagnosis, this must await further descriptions of splenic changes in this condition and the organization of criteria so that it may become diagnostic.

It is to be noted that our case resembles that of King¹ in that the onset of pain and shock was associated with defecation

Finally, it was of some interest in our case that evidences of central nervous system involvement as a complication of infectious mononucleosis were present

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INFECTIOUS LYMPHADENOSIS (MONONUCLEOSIS) AND THROMBOCYTOPENIC PURPURA, RECOVERY AFTER SPLENECTOMY

REPORT OF CASE

By WILLIAM DAMESHEK, M D , AND MICHAEL A GRASSI, M D

AMONG the features of infectious lymphadenosis (mononucleosis) which help to distinguish it from acute lymphatic leukemia is the lack of reduction in both red cells and platelets. Conversely, the association of well-defined anemia and/or thrombocytopenia with a marked degree of lymphocytosis in which abnormal lymphocytes are conspicuous, almost certainly indicates acute leukemia. The present case report deals with an exception to this rule. A young woman with severe purpura hemorrhagica was found to have generalized lymphadenopathy and a marked lymphocytosis. Although the clinical picture appeared to be that of acute lymphatic leukemia, the character of the lymphocytes suggested infectious mononucleosis, this was confirmed by a strongly positive heterophile agglutination. When the patient's bleeding became uncontrollable, splenectomy was performed and was followed by prompt recovery. The splenic histology was that of a reactive hyperplasia and that of adjacent lymph nodes was characteristic of infectious mononucleosis.

This case is of interest not only because of its rarity, but because it brings up anew the diagnostic features of infectious mononucleosis, the question of hypersplenism and its relationship to thrombocytopenia, and the lymphocytosis observed in a number of cases of idiopathic thrombocytopenic purpura.

REPORT OF CASE

Doris P., a married woman aged 22, first seen on September 28, 1944, complained of profuse catamenia of two weeks' duration. One week prior to the onset of this period, she had noticed profuse bleeding from the gums and small blood spots over the chest and arms. These increased in number. The menstrual bleeding was so profuse as to cause alarm. Further questioning revealed that in the past two years she had noted vaginal bleeding between periods, an increased tendency to bleed from cuts, and bleeding following a dental extraction. An occasional sore throat and a few swollen and tender glands in the neck had recently been noted. In 1940, a miscarriage of approximately two months' gestation had occurred, requiring hospitalization; the leukocyte count at this time was 26,500 with 80 per cent polymorphonuclears, no note regarding the platelets was made.

Examination revealed a pale, sick-looking young woman. Numerous petechiae in the conjunctivae and the buccal mucosae were present. The cervical and axillary lymph nodes were readily palpable as bean-sized, nontender glands. The edge of the spleen was felt two fingers' breadth below the left costal margin. Over the skin of the trunk and extremities were numerous petechiae and small ecchymoses. Profuse vaginal bleeding was present.

Blood studies at this time showed the following: Hemoglobin 80 per cent (Tallqvist) R B C 4,400,000, W B C 13,200, polymorphonuclears 24 per cent (band forms 14), lymphocytes 73 per cent (mature 61, young 12), monocytes 3 per cent. The red cells appeared normal but only a rare platelet was seen. The coagulation time was 6 minutes (normal), the tourniquet test strongly positive and the bleeding

From the J. H. Pratt Diagnostic Hospital and the Blood Laboratory of the Boston Dispensary and from the Hale Hospital, Haverhill, Massachusetts. Aided by grants from the Charlton Fund, Tufts College Medical School, and the Upjohn Company.

time more than 20 minutes (greatly increased). A tentative diagnosis of thrombocytopenic purpura was made and hospitalization advised. At first the patient refused. However, with continuing menorrhagia, her condition became critical and she was admitted to the Hale Hospital of Hingham, Massachusetts on October 2. At this time the hemoglobin was 60 per cent, R.B.C. 3,190,000, W.B.C. 12,800, polymorphonuclears 34, lymphocytes 64 per cent, platelets less than 10,000 per cu. mm. There was no clot retraction.

Therapy included a transfusion of 500 cc. of citrated blood, ascorbic acid 500 mg. daily, ferrous sulfate 1.0 Gm. daily, and menadione (vitamin K) 5 mg. daily. Since there was no improvement with these measures, she was seen in consultation by one of us (W.D.) at which time the generalized lymphadenopathy, the well-defined splenomegaly, and the great variability in lymphocyte morphology were pointed out as indicative of infectious mononucleosis. A sternal puncture showed an essentially normal marrow picture with islands of nucleated red cells and granulocytes, together with a slight increase in lymphocytes. There was no effacement of marrow architecture as seen in acute leukemia. The heterophils, although increased in number, showed a greatly diminished platelet production. The heterophile agglutination test was positive in a dilution of 1:640. The diagnosis of infectious mononucleosis in association with thrombocytopenic purpura was made, and the patient given a good prognosis. It was believed that the bleeding would probably diminish with subsidence of the infectious state.

However, vaginal bleeding continued unabated and the patient's condition grew rapidly worse. Despite another transfusion of 500 cc. of citrated blood, the hemoglobin on October 5 was 36 per cent and the red cell count 1,910,000. Since further transfusions seemed inadvisable, splenectomy was decided upon and performed under spinal anesthesia by Dr. Joseph Tartakoff. A transfusion of 500 cc. of blood was given 10 hours preoperatively. The spleen was found enlarged to about three times its normal size.

There was a dramatic postoperative response, with a rapid rise in platelets and a quick cessation of vaginal bleeding. The platelet count became increased immediately after operation and 36 hours later was 124,000 per cu. mm. There was a reversal in the leukocytic formula, which now became neutrophilic rather than lymphocytic. On the fifth postoperative day, lymph nodes were barely palpable. The heterophile agglutination test was positive in a dilution of 1:320. The peak platelet count was reached on the eighth postoperative day and was 600,000 per cu. mm. There was a gradual increase in hemoglobin and red cell count, which on October 18 showed the following: Hemoglobin 65 per cent, R.B.C. 3,310,000. During this 12-day postoperative period, an irregular fever of 98 to 102.2° F. was present but there was no evidence of pulmonary or other complications.

On October 19, the patient was allowed out of bed. Tablets of ferrous sulfate (1.0 Gm. daily) were again given. On October 31, the patient was discharged from the hospital feeling well and with the following blood counts: Hemoglobin 71 per cent, R.B.C. 3,940,000, W.B.C. 10,400, platelets 240,000, polymorphonuclears 52, lymphocytes 40 per cent, eosinophiles 6 per cent, basophiles 2 per cent. The heterophile agglutination test on October 16 was 1:40.

The spleen, which weighed 324 grams, was unfortunately placed in strong (100 per cent) formalin and then brought to Dr. H. Edward MacMahon for examination. Grossly, nothing abnormal was discerned. Histologically, the follicles were found enlarged, some with very large reactive centers showing extensive cellular necrosis and reticulum cell hyperplasia. The intersinusoidal pulp cells were increased in number. Slight subendothelial lymphocytic infiltration of the veins was present. Several moderately enlarged lymph nodes were present in the splenic hilus and were examined. The capsule and general architecture were well preserved. The cords were packed with unusually large numbers of lymphocytes. There was patchy and atypical hyperplasia of lymphoblast-like cells, among which mitoses were numerous. These encroached upon the cords and the sinuses and at one point spread out and filled the peripheral sinus. The findings in the lymph nodes were characteristic of infectious mononucleosis in the spleen with a simple reactive hyperplasia.

Comment. Infectious mononucleosis is a generalized infection of lymphoid tissue which is characterized by a benign, self-limited course, a characteristic blood picture, and the presence of a heterophile agglutinin in the blood serum. The blood picture presents a well-defined lymphocytosis, in which all types of lymphocytes are seen, including rare lymphoblasts, frequent very large lymphocytes with thick

glassy cytoplasm, and ordinary large lymphocytes, plasma cells, and mature lymphocytes. The term mononucleosis, suggesting as it does an increase in monocytes, is a misnomer, infectious lymphadenosis being preferable. An outstanding feature of the disease is the lack of associated anemia and of hemorrhagic phenomena. In fact, if anemia or thrombocytopenia is present, the diagnosis of acute leukemia rather than of an infectious condition is usually justified. However, a few cases have now been observed in which a well-defined thrombocytopenic purpura was present. We first observed it in a case subsequently reported by Tager.¹ The patient was a young female law student who was thought to have acute leukemia because of a severe hemorrhagic state in association with a high leukocyte count and the presence of numerous abnormal cells variously interpreted as myeloblasts, lymphoblasts, or monoblasts. The true nature of the process became apparent when the great variability in lymphocyte morphology characteristic of infectious lymphadenosis was pointed out. Other cases have been reported by Minot,² Magner and Brooks,³ and by Lloyd.⁴ In none of these cases was splenectomy required, complete improvement of the hemorrhagic disturbance occurring with subsidence of the infection.

In the present case, the history of a mild hemorrhagic condition of about 2 years' duration was present. However, a very marked accentuation in vaginal bleeding had occurred in the three weeks previous to her first visit, and spontaneously occurring hemorrhagic spots had developed during a two weeks' period. Thus, at least an acceleration in the bleeding disturbance had taken place simultaneously with the development of infectious mononucleosis.

The cause of this acceleration may be speculated upon. The spleen was considerably enlarged. Idiopathic thrombopenic purpura may well be of splenic origin and possibly in the nature of a form of hypersplenism. Studies by various investigators, including Frank,⁵ Limarzi,⁶ and Dameshek and Miller⁷ have indicated that the bone-marrow megakaryocytes, although increased in number, are deficient in platelet production. That this may be due to an inhibitory mechanism by an abnormal spleen acting upon the marrow is indicated by the greatly increased production of platelets by megakaryocytes which takes place very soon after splenectomy.⁷ Extracts of the spleen from cases of the disease have, in the hands of a number of investigators,⁸ including recently Dameshek and Denakes,⁹ resulted in the production of thrombocytopenic purpura in animals.

Splenomegaly from many sources is often associated with leukopenia and thrombocytopenia. Thus in chronic infectious splenomegaly (malaria, syphilis, tuberculosis, Felty's syndrome), in portal hypertension, splenic vein thrombosis, Gaucher's disease, etc., pancytopenia—anemia, leukopenia, or thrombocytopenia—is often present. Acute or subacute infections which involve the reticulo-endothelial system of cells and result in splenomegaly—typhoid fever, brucellosis, malaria, subacute bacterial endocarditis, kala-azar, etc.—are usually associated with leukopenia and thrombocytopenia. In the presence of well-defined splenomegaly in infectious mononucleosis, a similar state of hypersplenism might conceivably result, with the production of megakaryocytic inhibition and thus thrombocytopenia. In the present case, a possible mild thrombocytopenia had previously been

present, and the coincidental occurrence of infectious mononucleosis with considerable splenic enlargement might conceivably have aggravated the process. In any event, the development of severe bleeding jeopardized the patient's life and led to the operation of splenectomy, which was dramatically successful.

Of interest in connection with the present case is the occurrence in some cases of idiopathic thrombopenic purpura of a well-defined lymphocytosis. This has been pointed out by a few observers, notably by Minot,² who suggested the possibility of an altered endocrinal function. Another possible mechanism for the reversed granulocyte-lymphocyte proportion is hypersplenism. That is, the spleen might not only depress the formation and delivery of megakaryocytes and platelets, but cause a reduced delivery of neutrophils from the bone marrow and thus result in lymphocytosis. The whole subject of spleen-bone marrow relationships under normal and pathological conditions has only recently come to the forefront, and many questions relating to the possibly increased activity of the spleen must await further investigation.

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EXPERIMENTAL LEUKOCYTOSIS THE INEFFICACY OF P-CHLOROXYLENOL AND METHYL ACETAMIDE AS BONE MARROW STIMULANTS

By LEO M. MEYER, M D

INTRODUCTION

IN 1943, Zondek and Bromberg¹ produced leukocytosis in 25 human subjects with intramuscular injections of methyl acetamide and para-chloro-xylenol *. In this group, 17 were normal, 4 suffered from typhoid fever with leukopenia, and 4 had local infections with leukocytosis. The maximal responses were obtained by using a 70 per cent solution of methyl acetamide with 25 per cent p-chloro-xylenol (referred to as CXM). In the normal subjects, leukocytosis of 10 days duration resulted from injecting 50 cc of the above solution over a period of 3 days. In the typhoid fever patients larger doses were required to produce leukocytosis and this did not persist when the injections were discontinued. In the cases of local infections with leukocytosis, the response was more prompt, and reached extraordinarily high levels. In addition to the leukocytosis, there was a distinct shift to the left in the polymorphonuclear leukocytes with the number of young and band forms being increased. Sternal marrow examinations during the height of the leukocytosis showed a high percentage of band forms. The authors suggested that CXM had a selective stimulating action on the myeloid elements of the bone marrow without affecting the red cells or platelets. The graphs reveal that 5 days was the longest period of time over which the drug was given (in typhoid leukopenia). The authors also studied the effect of CXM on rats, hens and rabbits, but because of the marked fluctuations in the total number of white cells in these animals, no conclusions were reached.

METHODS

In this study the peripheral blood, bone marrow and internal organs of two groups of rats which were given doses of CXM comparable to those recommended by Zondek and Bromberg were investigated. Fifteen mature white rats (weighing about 200 Gm) and seventeen mature gray rats, averaging about 400 Gm, were used. The animals were housed in small cages in groups of 3 or 4 and given water and Purina dog chow ad lib. Because of the fluctuations in leukocyte counts in rats as a result of manipulation or trauma, the animals were handled several times a day, and blood counts taken (by clipping the tails) at different intervals, in order to determine the highest normal count for each subject. The intramuscular dosage was 0.1 cc for the smaller animals, and 0.2 cc for the larger ones. The rats were injected at intervals of 12 and 24 hours, and blood counts taken at varying periods. The smaller animals received a total dosage up to 0.4 cc, and the larger ones 0.8 cc. Several animals were found dead after 2 or 3 days administration of

From the Laboratories of the Carnegie Institution of Washington Cold Spring Harbor N. Y.

* Supplied by the Barrett Division of Allied Chemical and Dye Corporation

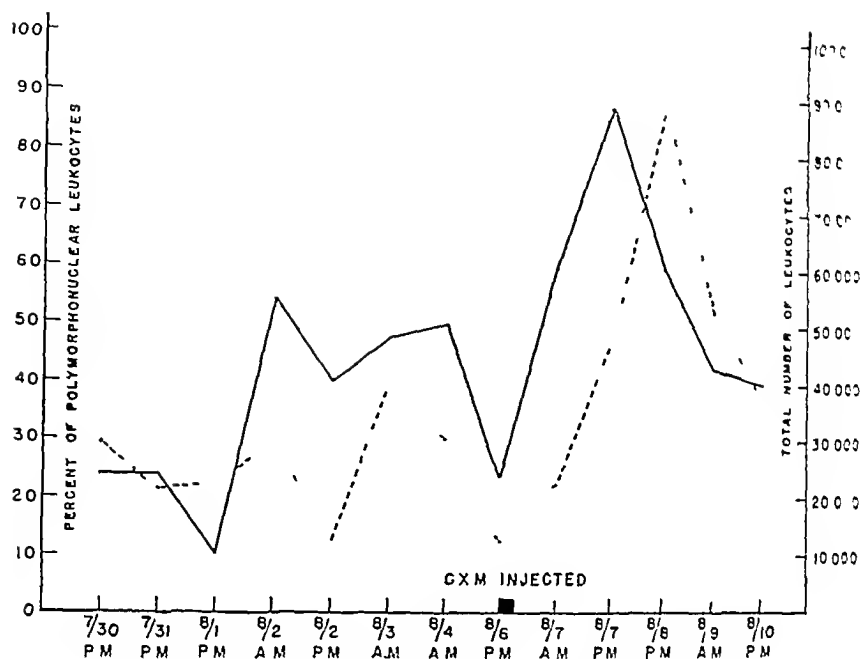


FIG 1 —, % PMN, ----, total WBC

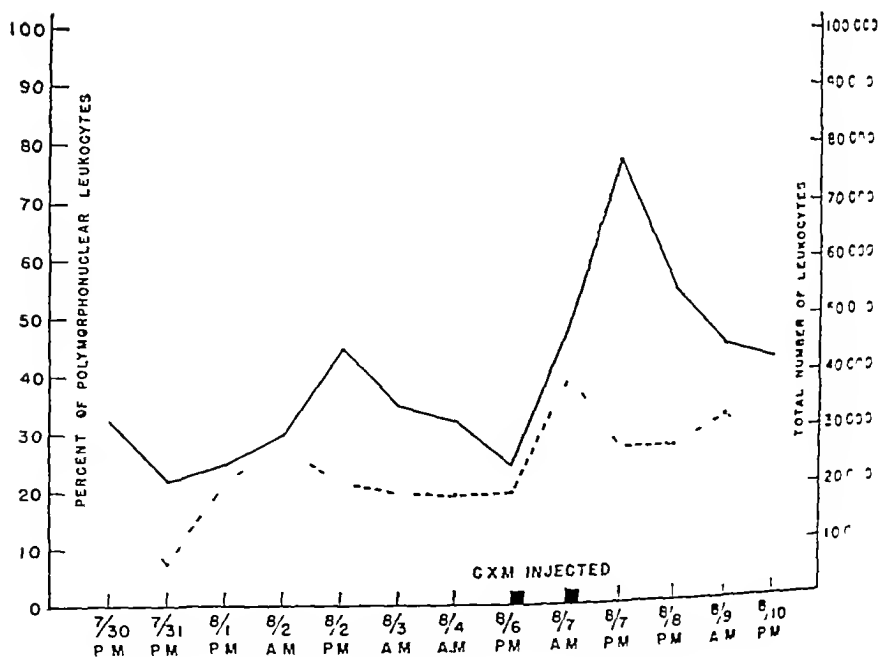


FIG 2. —, % PMN -- total WBC

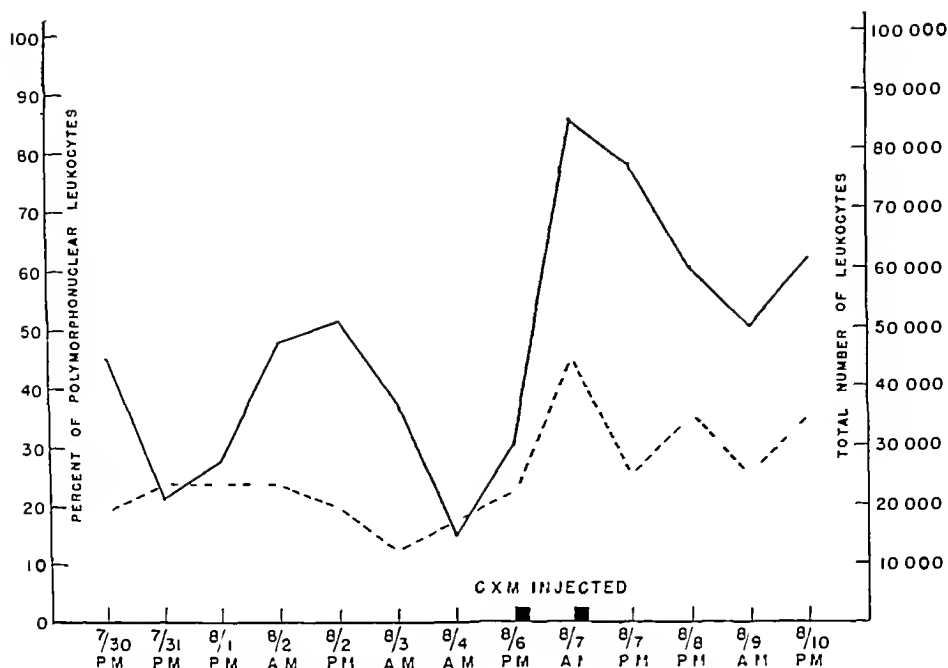


FIG 3 —, % P M N -----, total W B C

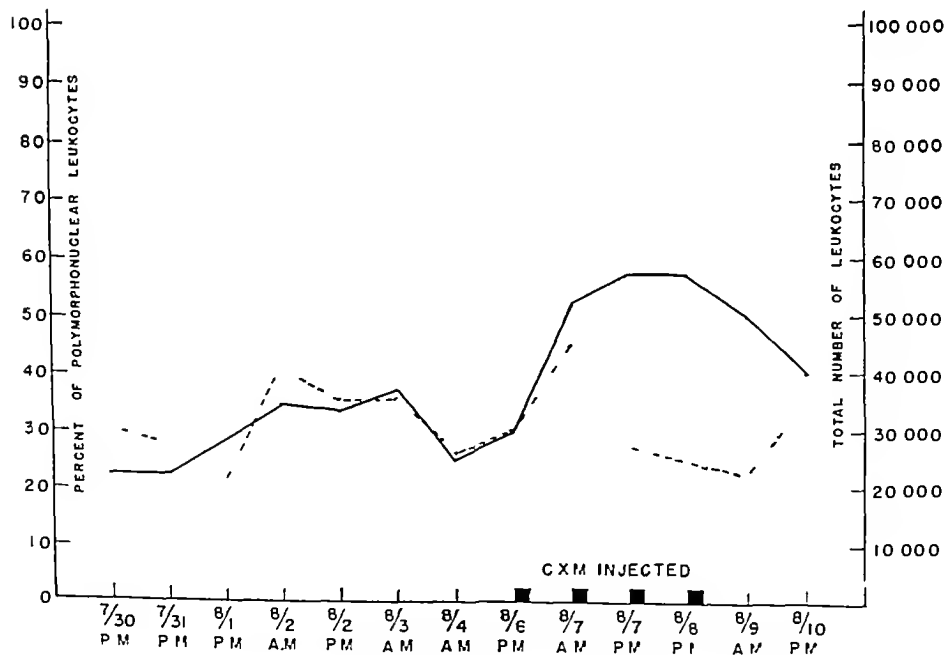


FIG 4 —, % P M N , -----, total W B C

the drug. The other animals were killed by a blow on the head. Smears of the vertebral marrow and histologic sections of a vertebra, liver and spleen were examined.

OBSERVATIONS

Figures 1 to 4 indicate the typical variations in leukocyte response to single and multiple injections of CXM in two series of rats. The maximal effect was reached 12 to 36 hours after the first injection, with a slow return to normal in 24 to 48 hours. The animals receiving 2, 3 and 4 injections evidenced a leukocyte response no greater or more substantial than those receiving one dose. In all instances, the maximal percentage of polymorphonuclear cells was about twice the highest figure reached in the week prior to the injections. All the cells were of the mature variety and no shift to the left could be determined. It is noteworthy that the animals responding with the greatest leukocytosis showed unusually high counts before parenteral therapy began. These observations are in accord with those of Zondek and Bromberg,¹ who noted hyperleukocytosis when CXM was injected in patients with local infections and leukocytosis. Bone marrow smears and sections in all 4 groups were normal. The myeloid elements were not hyperplastic, nor was there any suggestion of hypoplasia or atrophy of the erythroid series. A shift to the left did not occur and the megakaryocytes were unaffected. The sections of spleens also indicated normal histologic appearances in all 4 groups. Pathologic alterations occurred only in the livers of rats receiving single doses of CXM as evidenced by mild cloudy swelling, whereas those receiving multiple injections presented extensive and advanced hydropic degeneration. In some hepatic cells there was beginning fatty metamorphosis.

DISCUSSION

The increase in the number of polymorphonuclear leukocytes in the peripheral blood apparently results from a withdrawal of these cells from depots throughout the body (spleen, vascular bed and bone marrow) and not from marrow stimulation. The fact that the leukocytosis could not be sustained further suggests that there was no stimulation of granulocyte production but rather an expulsion of these cells from reservoirs. This redistribution phenomenon is a normal physiologic mechanism which in this instance is augmented by the injection of CXM. Apparently the capacity of the vascular recesses as storage depots for blood cells after delivery from the marrow is not exhausted by normal physiologic requirements. Examination of the bone marrow of human subjects during acute infections associated with leukocytosis usually shows an increase in the myeloid cells with a shift to the left. Such findings were not obtained in any of the rat marrow's observed.

SUMMARY

1. Single and multiple injections of CXM (methyl acetamide and p-chloro-o-xyleneol) have a similar action in increasing the total number of polymorphonuclear

leukocytes in the peripheral blood of a group of 32 rats. No shift to the left takes place.

2. There is no stimulation of the myeloid elements of the bone marrow to suggest that this leukocytosis is due to hyperplasia.

3. The evidence suggests that the mechanism for the leukocytosis produced by the CXM consists of releasing blood cells from depots in the body. This action is selective for the granulocytes as the red cells and platelets are not affected.

4. There is progressive degeneration of the parenchymal cells of the liver after single and multiple injections of these substances.

5. If less toxic combinations of methyl acetamide and para-chloro-xyleneol could be obtained, they might be of value in the treatment of certain patients with leukopenia or agranulocytosis more particularly when the bone marrow is hyperplastic but the granulocytes are not released.

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The author expresses his appreciation to Dr. Oscar Riddle for the generous provision of animal and laboratory facilities.

THE EXCRETION OF UROBILINOGEN IN THE STOOLS AND URINE DURING MALARIAL INFECTION

By A. DE VRIES, M.D.

With the technical assistance of F. Schiffer, Dipl. Ing.

A VARIABLE degree of blood destruction over and above that occurring physiologically is a constant phenomenon in attacks of malaria. Although blood destruction is always increased in a malarial attack, clinical signs of hemolysis (jaundice, anemia, etc.) are not always present.

The clinical syndrome of hemolytic anemia in malaria is known in its severest form as blackwater fever, which is characterized by the acute development of a marked anemia, hemoglobinuria, intense jaundice, and marked reticulocytosis. In less severe hemolysis, there is only slight jaundice and moderate anemia, accompanied by a slight rise in the number of reticulocytes and by an increased excretion of urobilinogen in the urine. However, in these cases the hemolytic nature of the jaundice is not generally accepted. Stitt^{2b} remarks: "It is not improbable, although this point is often disputed, that the yellow tinge of the skin and the sclerae often observed in malaria is due to the tinting of the tissues by the liberated hemoglobin and not as popularly believed to biliousness or cholaemia from bile absorption."

Hemolysis in its slightest form may at times be manifested, according to Stitt and Manson Bahr, by a positive indirect van den Bergh reaction in the blood, even without clinical jaundice. In addition, there are cases of malaria in which neither clinical signs nor laboratory findings pointing to hemolysis are observed. In this mild form, if treated early, no appreciable anemia develops, no jaundice is observed, no increase of bile pigments in the blood is found, even urobilinogenuria may be absent and reticulocytosis may not be encountered.

The purpose of this paper is to show that increased blood destruction in malaria is manifested by an increased excretion of urobilinogen in the stools, even when all the above recorded signs of hemolysis are lacking. These studies give further indication of the importance of the fecal urobilinogen output as a criterion of the degree of blood destruction.

MATERIAL AND METHODS

The material upon which this study was made comprises 10 consecutive cases of malaria. One patient suffering from benign tertian malaria was studied on two admissions, the second time during a relapse appearing 5 weeks after the first admission. The age of the patients varied from 19 to 67 years. Two were women, 7 men. None of the patients suffered from any other disease prior to admission, and no complicating conditions were present during hospitalization. In 6 cases the diagnosis was benign tertian malaria, in 4 malignant tertian. All the cases of

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benign tertian were relapses, and 2 of the patients with malignant tertian had their first attacks. In all of them the diagnosis was established by the finding of parasites in the blood.

The patients were admitted on the first to the sixteenth day after the beginning of the illness. All of them, except one, had fever either on admission or on the previous day. Only one patient (no. 3) was admitted 3 days after defervescence, nevertheless, significant observations on urobilinogen excretion could be made during hospitalization.

The number of paroxysms in the cases of benign tertian malaria varied from 1 to 3. Two patients with malignant tertian malaria had 2 and 3 paroxysms respectively, and 2 others had continuous fever. The total period in which fever occurred varied from 3 to 6 days in the patients with benign tertian malaria, and from 6 to 19 days in those with malignant tertian. Medication consisted of quinine and atabrin. Quinine as the only drug was given in 2 cases, atabrin as the only drug in 2 other cases. In 5 cases medication consisted of a course of quinine followed by atabrin. The maximal dose of quinine given was 11.4 grams in 9 days, the maximal dose of atabrin 2.8 grams in 7 days. No medication was given in case no. 3, as no parasites were found. In all cases asexual forms disappeared rapidly from the blood with medication. In only 2 cases (nos. 7 and 9) were gametocytes present after treatment.

Evidence of increased blood destruction was obtained by

1. Repeated determinations of hemoglobin and red cell values
2. Repeated determinations of the reticulocyte count
3. Repeated examination of the icterus index and the qualitative van den Bergh reaction in the serum
4. Quantitative determination of the amount of urobilinogen excreted in the stools and in the urine during and after the attack

The red cell and hemoglobin values were estimated in venous blood. The red cells were counted in a Spencer counting chamber, two determinations with two pipets were performed and the average taken. Duplicate values differing by more than 200,000 per mm^3 were discarded. The hemoglobin content of the whole blood was determined with the Stufenphotometer according to the method of Heilmeyer³ and expressed in grams per 100 cc.

The reticulocytes were examined by the Nile blue method. A smear of a 1 per cent aqueous solution of Nile blue was made on a slide and allowed to dry. A cover glass, on which was placed a tiny drop of blood, was gently pressed on the Nile blue smear and the count performed after 20 to 30 minutes. At least 500 red cells were counted. The reticulocytes were calculated as the percentage of the red cell count, as well as the total number per mm^3 (The normal value less than 1 per cent.)

The icterus index was measured by the modified Meulengracht method (the highest normal is 7).

The quantity of urobilinogen excreted in the stools and in the urine was determined by the method described by Watson.⁴ After discarding the first stool produced after admission, the stools were collected during 48 hour periods and were

examined for at least two periods (in only a few instances was one collection period continued for 72 hours). The samples were kept in a dark place in a container surrounded by ice. Determinations were performed at the end of each 48 hour period. The urine was collected during 48 hour periods under the same conditions. The values were expressed as milligrams of urobilinogen excreted in 24 hours. The normal values found by the author ranged from 50 to 180 mg. for the stools and up to 2 mg. for urine per 24 hours (5 normals). The normal values given in the literature range from 100 to 280 mg. per 24 hours (Dameshek et al.⁵ 66 to 180 mg. per 24 hours, the highest value given by Watson⁶ is 280 mg. per 24 hours).

RESULTS

THE TOTAL EXCRETION OF UROBILINOGEN PER DAY

In all of the 10 cases examined, the total excretion of urobilinogen (in stools plus urine) was found to be increased. The highest total daily excretions for each patient calculated from a 2 day period varied from 370 to 1142 mg. (431, 1142, 510, 370, 851, 438, 949, 367, 518, 477). The highest total daily excretions calculated from 4 day periods varied from 325 to 822 mg. (375, 822, 412, 314, 586, 361, 734, 325, 475). Thus in 9 cases the excretion was found to be increased during a continuous period of at least 4 days, during which two 48 hour samples were examined. This excludes accidental fluctuations. (In case 9 the total excretion could be measured only during a 2 day period at the beginning of the hospitalization.)

Factors which might possibly influence the excretion of urobilinogen are diarrhea and constipation, diet and fever. None of our patients had diarrhea or severe constipation, and none received laxatives. Since ingestion of a large quantity of fat may increase hemolysis⁷ all of our patients were given a normal diet with an average fat content of 60 grams a day. In the writer's experience fever itself does not increase the quantity of urobilinogen excreted in the stools (normal values were obtained in Malta fever and typhoid fever). This corresponds to the findings of Vaughan and Saifi,⁸ who generally did not find an increased excretion of urobilinogen in infectious diseases. Medication cannot be held responsible for the increased excretion, since during the course of the treatment the excretion of urobilinogen gradually diminishes. In case no. 3 no medication was given at all and the urobilinogen excretion in the stools was found to be increased.

It is thus evident that the increase in the total excretion of urobilinogen observed in our cases can only be the sequel of an increased red cell destruction due to the malarial infection.

The earliest period in which the total daily excretion of urobilinogen could be measured was the third and the fourth days of the illness (case 1). Although this patient came in within 24 hours after the beginning of the attack, the necessity of discarding the first stools made the determination possible only on the third and the fourth days, when it was found to be increased (319 mg. per 24 hours). An increased total daily excretion of urobilinogen was observed 2 to 7 days after defervescence. In 6 cases a return to practically normal values was observed on the seventh to twenty-first day (nos. 1, 2, 4, 6, 9, 10). In two cases (5, 8) the last determinations on the eleventh and ninth days still showed an increased value.

Initials	Age	Sex	Malaria	No of paroxysms	Days of fever	Highest fever degree Celsius	Parasites present on day of illness	Therapy			1st count			Lowest values observed				Fall in			Highest reticulocyte count			Jaundice				Urobilinogen excretion per 24 hours in milligrams			
								Drug	Dose in grams	On day	Red cells in millions/mm ³	Hemoglobin in gram %	On day	Red cells in millions/mm ³	Hemoglobin in gram %	Red cells in millions/mm ³	Hemoglobin in gram %	In days	%	Total per mm ³	On day	Day of illness	Clinical jaundice	Icterus index	Van Den Bergh reaction		On days	In stools	In urine	Total	
																									from till	On day					
D F	67	F	Vivax	1	4	39.5	+1 -5	1.8 1.6	3 1	43 11	6 1	10 11	6.0 33		3	6	246 000	6	2 5 12	1 1 5	1 1 1	1 1 1	3-4 5-6 7-10 9-10	271.48 423.7 151	319 7 3	319					
J M	32	M		3	5	40.2	+5 -8 -10	6 2.1 9.15	5 5	35 12	4 11	4 9	11 3.0	41 1	6	1	65 500	14	6 8 11	1 1 7	1 1 1	traces 1 1	6-7 8-10 11-12	495 116 211	7 5 50	502 142 246					
Sh K	21	M		2	3	40	+1		7 4	69 13	2				<1	<47 000	5-12	9	1	5	1	6-9 9-10	105 503	7 10	315 510						
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M L	28	M		3	5	39.2	+5 -8 -10	3.0 1.5 8.12	6 4	44 12	9 10	4 11	13 3			<1	<41 000	6-10	5	15	1	traces	6-7 8-9 10-11	875 26 310	26 8 10	951 320					
M I	28	M		3	5	40	+3 +6 -12	10 8 3	9 4	5 16	14 8	9 4	48 13	60 68	1.2 5	<1	<57 000	3-10	6	10	1	1	4-6 7-8 9-10	420 291 235	17 10 5	438 295 216					
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but there was a definite tendency to become normal. These findings furnish additional proof that the increased excretion of urobilinogen found in malaria is due to the infection itself. In cases 3 and 7 the last values obtained were still high but it may be assumed that a return to the normal would have been found had the examination been extended for a longer period.

The amount of urobilinogen excreted in the stools forms, of course, the major part of the total excretion. The highest daily excretion in the stools, calculated as the average of a 2 day period excretion, was 1136 mg.

THE EXCRETION OF UROBILINOGEN IN THE URINE

In all cases except one (9) the daily excretion of urobilinogen in the urine was found to be significantly increased. The highest daily excretion observed in a 2 day period was 58 mg. The amount of urobilinogen excreted in the urine returned to practically normal values in 3 cases (1, 7, 8) after about 8 days. In 3 cases (4, 5, 10) the excretion decreased markedly but did not reach the norm during 10 to 13 days. In cases 2, 3, and 6 there was also a tendency to decrease but to a lesser degree. The decline in the excretion of urobilinogen in the urine preceded that in the stools. There was no correlation between the excretion of urobilinogen in the stools and in the urine. For example, on comparing the excretions in cases 1 and 9, the quantities of urobilinogen excreted daily in the stools were 423 (case 1) and 516 mg (case 9), and in the urine 48 mg (case 1) and 45 mg (case 9) respectively.

ANEMIA

Although increased blood destruction was present in all cases examined, significant changes in the red count were observed in only 6 cases (1, 2, 5, 6, 9, 10). An appreciable fall in the red cell and hemoglobin values during the period of study was observed in only 3 cases (6, 7, 10). There was no correlation between the total quantity of urobilinogen excreted and the fall in the red cell and hemoglobin values. Sufficient explanation for this is seen in the fact that the level of the red blood cell values before the beginning of the illness could not be determined, that changes in water balance may mask actual changes in the blood count, and finally that an increased blood destruction may be compensated by increased regeneration.

No correlation was found between the degree of anemia and the amount of urobilinogen excreted in the urine (compare cases 9 and 4).

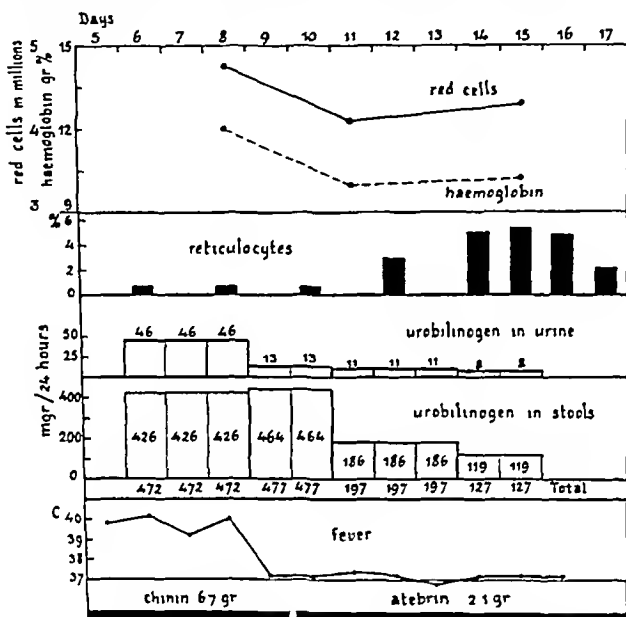
JAUNDICE AND BILE PIGMENTS IN THE BLOOD

In only 1 case (no. 1) was definite jaundice noted clinically, corresponding with a high icterus index (50) and a positive indirect van den Bergh reaction. In the other 9 cases clinical jaundice was not observed, and in none of them was the icterus index higher than 20, the indirect van den Bergh reaction was positive in only 1 of these cases. In 4 cases only traces of indirect reacting bilirubin were found in the blood. In the remaining 4 cases no laboratory findings suggestive of latent jaundice were revealed. It is thus evident that there is no correlation between the total amount of urobilinogen excreted and the degree of jaundice, the height of the icterus index or the presence of an indirect van den Bergh reaction. Nor is there a

relationship between the amount of urobilinogen excreted in the urine and the laboratory evidence of jaundice

RETICULOCYTES

An increased number of reticulocytes was found in only 3 cases (1, 9, 10) with the lowest red cell values. The increased excretion of urobilinogen was not associated with reticulocytosis (cases 7, 2) in the absence of anemia. It seems that in some cases the appearance of reticulocytosis may be delayed by the malarial in-



fection. In case 10, for example (see graph), the reticulocytes began to rise 3 days after defervescence and after the disappearance of the parasites from the blood. This corresponds to the observations of Castle and Minot⁹ and of Blackie¹⁰. However, reticulocytes may also appear while fever is still present and parasites are found in the blood (case 9).

DISCUSSION

In contrast to the numerous reports on the excretion of urobilinogen in the urine in malaria, studies on the total excretion (in stools and urine) are scarce.

Manson Bahr¹¹ states: "The corresponding pigment in the faeces (hydrobilirubin) is increased to twenty times the normal amount, as long as there is fever and parasites are present in the blood."

Eppinger¹² mentions a case of relapsing malignant tertian malaria in which the daily amount of urobilinogen excreted in the stools was 330 and 364 mg (the upper normal range according to this author is 140 mg daily).

An increased daily total excretion of urobilinogen was found in all our cases. This excess of excretion was present even when no appreciable anemia or other sign of hemolysis was observed. Although the amount of excreted urobilinogen

cannot be used as an exact indication of the quantity of red blood cells destroyed and the observed lack of correlation between jaundice and total urobilinogen excretion leads to the assumption that the amount of red blood cells destroyed is not the only factor responsible for hemolytic jaundice in malaria. As pointed out by Miller, Singer, and Dameshek,⁸ none of the various indices of increased blood destruction is specific, except for the fecal urobilinogen output, which gives unequivocal evidence of an increased breakdown of blood.

The additional factor responsible for the development of jaundice in hemolytic conditions is probably the inability of the liver to deal with the bilirubin formed (Watson¹²). The liver in malaria was studied systematically by some investigators, and a disturbance in liver function was revealed in most cases (Kopp et al.⁹ Greene et al.,¹⁰ Mirsky et al.¹⁶).

No attempt was made in our study to correlate jaundice and routine liver function tests as they bore no constant relationship to the state of the liver. Some indication of the functional capacity of the liver may be furnished by the amount of urobilinogen excreted in the urine (Watson¹⁷).

Numerous authors are of the opinion that the amount of urobilin or urobilinogen in the urine is always increased in malaria (Atkinson,¹⁸ Antic and Neumann¹⁹ Saupe,²⁰ Reynolds,²¹ Gordon,²² Ballerstedt²³). Other investigators claim that the amount of urobilinogen in the urine is increased in some cases of malaria and not in others (Uvedale Owen,²⁴ Plehn,²⁵ Starr²⁶). Uvedale Owen²⁴ states that urobilinogenuria is generally more excessive in malignant tertian malaria and that the quantity of urobilinogen excreted in the urine bears no relationship to pyrexia, enlargement of the spleen, or the number of parasites present in the blood. His studies indicate that quinine increases the excretion of urobilinogen in the urine and that this increase may appear 8 to 37.5 hours after the first dose.

According to Plehn²⁵ urobilinuria in malaria is only a symptom of liver disturbance. Hence urobilinuria need not necessarily be found in every case of malarial fever, for malaria may exceptionally spare the liver. The results of our quantitative determinations show that the excretion of urobilinogen in the urine in malaria may or may not be increased. In 7 of 10 cases it was found to be markedly increased during the period of fever and decreased rapidly after defervescence. In 1 case (9) it was found to be practically normal, although fever was present and parasites were found in the blood. In 2 cases there was a slight increase of urobilinogen excretion in the urine (cases 2 and 3).

No correlation was seen between the amount of urobilinogen excreted in the urine and that excreted in the stools. It thus seems that red cell destruction alone cannot account for the increased excretion of urobilinogen in the urine and that a disturbance in the capacity of the liver to remove the absorbed urobilinogen from the intestine must be assumed. In this respect the bile pigments in malaria behave as in other hemolytic diseases (Watson¹⁷). Fever itself cannot be held responsible for the urobilinogenuria, as normal values were found in case 9 in spite of hyperpyrexia and the same observation was made in other febrile diseases such as malaria fever and typhoid fever (see also Watson⁶). No difference was found between

benign and malignant tertian malaria as to the quantity of urobilinogen excreted in the urine. On the other hand, the excretion of urobilinogen in the feces is constantly increased.

SUMMARY

In 10 cases of malaria (6 benign tertian, 4 malignant tertian), the excretion of urobilinogen in the stools and in the urine was studied. In all 10 cases the amount of urobilinogen excreted in the stools was found to be increased. After defervescence and disappearance of parasites from the blood the excretion gradually declined. The increased excretion of urobilinogen in the stools was *the constant and sometimes the only evidence of increased blood destruction* occurring at times in the complete absence of jaundice and reticulocytosis. Increased excretion of urobilinogen in the urine was not a constant feature.

It is suggested that the development of jaundice and of urobiligenuria is due not only to the liberation of pigments by the hemolysis, but to a disturbance in the liver function.

This study lends further confirmation to the concept that the only unequivocal evidence of increased blood destruction is shown in an increased output of urobilinogen in the feces.

The author is indebted to Dr. M. Rachmilewitz for his suggestions and criticisms.

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EDITORIALS

FOLIC ACID

WHAT is known as folic acid appears to have become one of the wonder drugs of 1946. A great variety of widely scattered and often unrelated investigations by biologists, chemists, pharmacologists, and clinicians has culminated in the startling fact that only a few milligrams of this chemical are productive of reticulocyte and erythrocyte responses in pernicious anemia. Even more striking are the effects in such related deficiency states as sprue, in which the response to the injections of liver extract, although good, is often slow and far from satisfactory. In other macrocytic anemias originating in a deficiency state (pernicious anemia of pregnancy, tropical macrocytic anemia, perhaps even the macrocytic anemia of cirrhosis of the liver), it seems likely that folic acid may supply a missing factor. It should be realized, however, that not all macrocytic anemias are due to a deficiency state, e g, certain cases of leukemia, hemolytic anemia, bone-marrow disease, etc, may be associated with a mean red cell size above the average. In this issue, Berry and Spies bind all the various loose threads—the *L* cases factor, the M factor, the B₁₂ vitamin, etc—into an impressive meshwork representing the present state of our knowledge of this interesting chemical. A host of new problems for the active investigator is immediately set up. What relationship, if any, does folic acid have to liver extract, since highly purified preparations of the latter material contain insufficient folic acid to result in a remission? How does it work in pernicious anemia—directly, or through some complex set of enzyme systems? Will it prove as beneficial as liver extract in preventing and treating the neurologic features of pernicious anemia? etc, etc. Until very recently this disease seemed to be all set, signed and delivered, now, its relation to the other deficiency macrocytic anemias has to be redefined, and it again becomes a fresh and fertile field for the investigator.

NOT SO BENIGN INFECTIOUS MONONUCLEOSIS

Infectious lymphadenosis, or mononucleosis as it has been miscalled for many years, has always been considered a very benign condition. Certainly this is almost always the case. In this issue, however, are three articles which stress some of the occasionally serious complications, such as spontaneous rupture of the spleen, which may ensue. The moral of the articles by Smith and Custer and by Vaughan, Regan, and Terplan is plain. In a patient known to have the disease, splenic palpation may well be dispensed with, if the disorder is only suspected, go gently! Apparently the surface of the organ is rendered so friable by the disease that the slightest trauma may be just sufficient to cause its rupture.

Another more unusual complication is the development of thrombocytopenic purpura due perhaps to hypersplenic effects. In the case cited in this issue, splenectomy finally became necessary to control the bleeding. Other complications may be hepatitis, severe Vincent's angina of the throat, and various acute disorders of the central nervous system. The lack of benignity under all these circumstances suggests that further search should be conducted for an appropriate form of therapy.

WILLIAM DAMESHEK, M D

ABSTRACTS

HEMATOPOIETIC TISSUES

OLIVER P. JONES, Ph.D

GROWTH OF LYMPH NODES, THYMUS AND SPLEEN, AND OUTPUT OF THORACIC DUCT LYMPHOCYTES IN THE NORMAL RAT *W O Reinhardt Anat Rec 94: 197-211, 1946*

The results of studying growth of lymphoid tissues in 474 rats from birth to 19 months of age are very interesting, but most important for the hematologist are the data collected on the output of lymphocytes. Thoracic and cervical lymph ducts in 33 adult female rats were cannulated for varying periods and the lymph collected for cell count and volume studies. The total number of lymphocytes delivered to the blood stream per hour is about 9 million. This means that approximately 10 per cent of the total lymphocytes in the blood stream are replaced hourly. The entire lymphocyte population in the blood is replaced 2.05 times per day and the average life span of a lymphocyte in the blood stream is 1.2 hours. In this connection it should be noted that following transfusions of cells labeled with acridine hydrochloride, lymphocytes were found chiefly in the bone marrow (Farr *Anat. Rec. 94: 460 1946*).

BLOOD TRANSFUSIONS AND BLOOD SUBSTITUTES

EUGENE L. LOZNER, M.D

AMINO ACIDS IN THE PRODUCTION OF PLASMA PROTEIN AND NITROGEN BALANCE. *S C Madden and G H Whipple Am J M Sc 211: 149-56, 1946*

It is obvious from this and previous reports of Madden and his various co-workers that the essential amino acids may be given by mouth, vein, subcutaneously or intraperitoneally and may maintain nitrogen and weight equilibrium in the dog. The observations reported herein include those on 3 patients in which amino acids were given intravenously, subcutaneously and orally with favorable clinical results. Madden feels that amino acid mixtures are better tolerated parenterally and more palatable orally than protein hydrolysates. However, he points out that amino acids offer practical problems in large scale production and as yet are expensive. At the present moment they cannot be considered a significant therapeutic agent except in research laboratories.

STUDIES ON TRAUMATIC SHOCK. V. THE TREATMENT OF CLINICAL SHOCK WITH GELATIN. *E I Evans and H S Rafal Ann Surg 121: 478-94, 1945*

Evans and Rafal in this article provide additional data in support of the increasingly evident conclusion that gelatin is a very useful substitute for plasma in situations where decreased plasma volume is present or is imminent. They have used both lightly and heavily degraded gelatin in traumatic shock and burn shock, and in these situations the lightly degraded gelatin appeared to be superior in that it was retained longer in the blood stream. They discuss the advantages and disadvantages briefly and it may be well to repeat the disadvantages here. These consist of pseudo-agglutination of the red cells complicating blood grouping and cross matching (this may now be abolished by using a drop of 1 per cent gelatin in the erythrocyte-serum gelatin suspension), high viscosity which renders the gelatin solutions impossible to administer when cold (a 6 per cent gelatin solution must be kept at approximately 35° C.) and the fact that gelatin does not supply any oxygen-carrying capacity which may be important both in proper management in shock due to trauma and in burns. Thus they point out that gelatin (as well as plasma) cannot be used as a true substitute for whole blood.

This reviewer desires to call attention to two additional disadvantages: one the nutritional inferiority of gelatin as a protein and the other the fact that these gelatin solutions require especially careful preparation in order to produce a nonpyrogenic and uniform material.

HEMOSTASIS AND HEMORRHAGIC DISEASES

MARILYN T. SCHITTONE, M.D.

THE PROGNOSTIC VALUE OF MARROW EOSINOPHILS IN THROMBOCYTOPENIC PURPURA *Steven O. Schwartz*
Am J M Sc 209 579-87 1945

The author presents a study of 30 cases of thrombocytopenic purpura and attempts to show that increased eosinophils in sternal bone-marrow puncture preparations represent a favorable prognosis for spontaneous recovery while the presence of relatively few such cells suggests a chronic course with probable necessity for splenectomy. He believes that marrow eosinophilia under such circumstances represents an allergic state and that the thrombocytopenia represents a sensitization reaction involving the megakaryocytes. An arbitrary base line of 50 eosinophils per 1000 leukocytes of the metamyelocyte and polymorphonuclear series was chosen because eosinophils presumably arise from the same stem cell and because specific stimulation or decrease of granulocytes does not occur in this disease. Those patients showing more than 50 eosinophils per 1000 granulocytes in bone marrow had a relatively benign course followed by complete hematological and clinical recovery without splenectomy. Those with fewer than 50 eosinophils per 1000 granulocytes tended to have protracted courses without cure except by splenectomy. Blood transfusions were felt to hasten recovery in the first group. Correlation was not found between marrow and peripheral eosinophilia.

The author is aware of the limitations necessarily inherent in attempting to make a prognosis based on the numerical evaluation of a single cell, but suggests that certain correlations exist which may be helpful in considerations of prognosis and treatment.

Case Analysis: 12 cases 50 or more eosinophils per 1000 granulocytes in marrow, had short courses with spontaneous recovery 1 case. Less than 50 eosinophils, spontaneous recovery (the author feels the marrow was examined too late and after platelets had increased) 2 cases. Few eosinophils and chronic course 5 cases. Few eosinophils, and responded to splenectomy 3 cases. High eosinophils and responded to splenectomy (allergic thrombopenia apparently can do well with splenectomy) 4 cases. Few eosinophils, and death of patient 3 'equivocal' cases.

OPTIC ATROPHY AFTER THROMBOCYTOPENIC PURPURA *P. V. Carelli and J. Cangelosi* *J. A. M. A. 129 550, 1945*

A case is presented of a 6 year old Italian male who was first seen because of uncontrollable, nontraumatic epistaxis occurring one week after the onset of an upper respiratory infection. Examination revealed pallor and hemorrhagic areas over the eyes, forehead, lips, and lower extremities. Laboratory data disclosed a mild normochromic anemia, leukocytosis, and marked thrombocytopenia with normal bleeding and clotting time. Two weeks after onset of illness, he suddenly developed failing vision. A diagnosis of left corneal infiltrate and left retinal hemorrhage was made. Under therapy the infiltrate healed but subsequently optic atrophy developed. Splenectomy, performed 7 weeks after onset of illness and during an apparent quiescent phase, produced an almost immediate return of the blood picture to normal but the eye findings are still unchanged 20 months after onset of the disease. The bilateral optic atrophy is attributed to hemorrhage into the nerve sheath and probably into the orbit. A review of the literature tends to show the rather infrequent observation of a choked disk and of 2 cases of optic atrophy which did not respond to splenectomy.

This case emphasizes the necessity for the consideration of early splenectomy in cases of idiopathic thrombocytopenic purpura especially when massive or uncontrollable bleeding occurs. Prolonged observation may result in a serious and irreversible complication or even in death from hemorrhage into a vital organ.

THROMBOCYTOPENIC PURPURA DUE TO SODIUM SALICYLATE *Major A. E. Rappaport, Lt Col C. E. Nixon, and Major W. A. Barker* *J. Lab. & Clin. Med. 30 916 1945*

The authors present the case of a 37 year old Negro male who was treated for rheumatoid arthritis with a total of 44 grains of sodium salicylate over a period of one month without response. Treatment was reinstituted 3 weeks later for 9 days with a daily dosage of $\frac{1}{2}$ gram a day following which a severe epistaxis occurred. Treatment was continued two days more with the development of hematuria, hemate-

mesis large oral and conjunctival ecchymoses and subsequently large bladder hematomas and extensive petechiae. Platelet counts were decreased (normals for the laboratory were not given). The tourniquet test was strongly positive. There was poor to absent clot retraction and the bleeding time was slightly prolonged. The other determinations were normal or consistent with the severe anemia. A bone marrow puncture specimen revealed megakaryocytes which appeared to be plentiful and which were immature with vacuolated cytoplasm and pyknotic nuclei. Because the diagnosis of purpura hemorrhagica was made, a splenectomy was performed but the patient died 20 days postoperatively. Examination of the spleen revealed metaplastic foci of megakaryocytes.

The authors feel that this case represents one of allergy to sodium salicylate with the production of thrombopenic purpura because of the history of exclusive medication with sodium salicylate, the failure of splenectomy to cure and the morphological data revealing the toxic effect represented in the megakaryocytes.

The extreme reaction evidenced in this case contradicts Schwartz's impression that allergic thrombocytopenia tends to show recovery whether or not splenectomy is performed. This case may represent one of extreme toxicity rather than one of simple allergy where recovery ordinarily follows promptly upon removal of the offending allergen. No note is made of a marrow eosinophilia.

THROMBOCYTOPENIC PURPURA DUE TO MAPHARSEN *Marrin Schwartz and Elmore C. Vonder Heide* J. A. M. A. 188 657-59 1945

A case is reported of thrombocytopenic purpura following the prolonged administration of mapharsen. A 24 year old white male was treated for early syphilis with 0.06 Gm. of mapharsen and bismuth for 30 weeks. Two days following the last dose, he developed a severe headache, nausea and vomiting, bleeding from the mouth and nose, and petechiae and ecchymoses of the extremities. Laboratory studies revealed a mild anemia, marked reduction in platelets, normal clotting time, prolonged bleeding time, no clot retraction, and a strongly positive capillary fragility. The spleen was enlarged to two fingers below the costal margin. Recovery ensued rapidly on withdrawal of the drug. A month later test dosing with mapharsen (containing 1.5 mg. of arsenic) produced severe headache but no change in platelet count. However, 40 mg. of neoarsphenamine (containing 8 mg. arsenic) administered intravenously reduced the platelet count from 180,000 to 1,800. Within one half hour after the injection the patient began to bleed from the nose and mouth and hospitalization was necessary. Laboratory data again show the findings of thrombocytopenic purpura. Three days later all findings were normal. Bismuth therapy was reinstituted following hospital discharge with no untoward effect. This reaction to mapharsen has many of the characteristics of a sensitivity reaction. The exact mechanism is not understood. Thrombocytopenic reactions to arsenicals in over 60 cases culled from the literature were all characterized by free bleeding from mucous membranes, a rapid fall in circulating thrombocytes (as rapidly as 15 to 30 minutes following the injection of arsenical), and evidence of increased capillary fragility (positive Rumpel-Leede test and purpuric spots). In all of the cases the bone marrow showed no abnormality and in most cases the platelets in the blood were promptly increased following injection of epinephrin. The platelets in most cases begin to rise in 24 to 48 hours and return to normal in 4 to 7 days. No fatalities are reported as a result of this complication. There does not appear to be a selective destruction of the megakaryocytes in the marrow and the authors feel that the platelets may simply be diverted temporarily into dilated and stagnant capillary beds.

The significantly normal megakaryocytes in the bone marrow suggest that this type of thrombocytopenic purpura is more truly the result of a shock reaction.

THROMBOCYTOPENIC PURPURA FOLLOWING THE USE OF SULFATHIAZOLE *P. S. Strory and E. M. Glassberg* Ann. Int. Med. 23 237 1945

Thrombopenic purpura following sulfonamide therapy is a rare but dangerous occurrence. It may be the result of acquired sensitivity or it may represent a true idiosyncrasy following the initial use of the drug. The authors present the case of a 19 year old soldier who was treated with sulfathiazole for ear infection. After 19 grams of this drug had been given he developed a full blown picture of thrombocytopenic purpura with severe epistaxis, widespread petechiae and ecchymoses throughout the skin and visible on mucous membranes, prolonged bleeding time, normal clotting time, poorly retractile clot formation and a marked reduction in platelets. Intensive therapy with transfusions, vitamins C and K, and calcium was

given and after a prolonged course of illness, followed by most of scarlet fever's complications, the patient recovered. The authors could find no references in the literature to the development of purpura following scarlet fever only.

HEREDITARY HEMORRHAGIC TELANGIECTASIA *G. Q. Voyles and James O. Ristby* *Ann Int Med* 22: 730-36, 1945

The authors present 2 cases of hereditary hemorrhagic telangiectasis with the characteristic triad of multiple telangiectases, hemorrhage or anemia, and a history of familial occurrence. The extreme fragility of the lesions and the time of their appearance largely determine symptomatology. As the nasal mucous membrane is usually involved first, epistaxis occurs with increasing frequency from late childhood onward. In middle life, the skin and visceral lesions appear and add to the blood loss. The resulting anemia is often severe but the hemoglobin is seldom below 50 per cent. Skin lesions occasionally disappear after a period of years but the anemia persists. As the patient ages, the quantity of blood lost increases and nasal hemorrhages of 1000-1500 cc. are not uncommon. The 6 per cent mortality of the disease is associated with these conditions. Over 500 cases occurring in 100 families have been described in the literature. The hereditary factor seems to be transmitted as a simple dominant by and may affect both sexes. Atavism may occur.

Although various forms of treatment have been advocated, none seems to be satisfactory. However, cauterization, radium packs, iron, and transfusions are used.

The authors' patients—men of 51 and 70—both had hemoglobin levels lower than those usually described and lesions in the skin, mucous membranes, upper respiratory tract, and colon. One case had microscopic hematuria on several occasions.

The authors make the point that in cases of recurrent epistaxis or chronic anemia for which no cause can be ascertained, hereditary hemorrhagic telangiectasia should be considered and a search made for the lesions in the nasal, oral, and pharyngeal mucosa. The presence of telangiectases in the skin and a familial history of similar lesions or epistaxis complete the diagnosis.

(Recently the use of thrombin fibrin foam has been of value in controlling local bleeding, particularly from the nose.)

HEREDITARY TELANGIECTASIA *Wayne Rundles* *Am J M Sc* 210: 76-81, 1945

The author reports a case of a 56 year old white male with hemorrhagic telangiectasia (probably similar to the atavistic type of atypical cases without positive familial history) who had suffered from repeated epistaxis from age 14 and in later life from gastro-intestinal hemorrhage severe enough to produce an incapacitating anemia. Multiple gastric telangiectases were seen by gastroscopic examination. An aneurysm of the pulmonary artery was present which did not increase appreciably in size during 7 years of observation. Aneurysm of the pulmonary artery had not been previously reported. A unique feature at autopsy was the presence of multiple hazelnut-sized aneurysms of the splenic artery.

CONGENITAL AFIBRINOGENEMIA Report of a case with review of the literature *J. L. Henderson, G. M. M. Donaldson, and Harold Scarborough* *Quart J Med* 14: 101-12, 1945

A case of congenital afibrinogenemia in a boy of 11 is presented, and 6 other cases recorded in the literature are reviewed. The principle features of the disease are its hereditary character, a high incidence of consanguinity in the parents, the susceptibility of both sexes, a total absence of fibrinogen in the blood, complete incoagulability of the blood, a usually prolonged bleeding time, a great reduction in capillary resistance, a low erythrocyte sedimentation rate, and in some cases intermittent thrombocytopenia. The absence of fibrinogen is regarded as the principal cause of the hemorrhagic diatheses, but diminished capillary resistance may be a contributory factor. The principle distinctions between congenital afibrinogenemia and hypofibrinogenemia are discussed. The greatest differences occur in the clotting time; it is normal in the latter condition whereas no clotting occurs in the former. The authors conclude that from the literature it would appear that congenital fibrinogenopenia is caused probably by some hereditary defect of fibrinogen formation whereas the acquired type is caused by some toxic or neoplastic interference with fibrinogen formation. When acquired, the condition is sometimes transitory. It is thought that the liver and bone marrow are concerned with the function of fibrinogen formation.

HEMOPHILIA LIKE DISEASE IN THE FEMALE (with a note on the clotting time of the recalcified plasma)
Frederick W. Madison and Armand J. Quick Am J M Sc 209 443-47 1945

A case of a 30 year old female resembling hemophilia both clinically and in laboratory behavior presented showing bleeding into muscles, intermittent hematuria, normal bleeding and clotting times, prothrombin concentrations and platelet counts with terminal hemorrhage at base of tongue. Coagulation was markedly delayed (17-21 minutes by Lee-White method). Madison and Quick compared this case and true male hemophilia and find them alike except for age of onset and clotting time of recalcified plasma. Oxalated hemophilic plasma subjected to high centrifugation clots significantly more on recalcification than that obtained by spontaneous sedimentation or slow centrifugation. This could not show that difference. Armand J. Quick (in Am J M Sc 202 1941) showed that the test was consistently positive in a small series of true hemophilia cases but negative in one other atypical or hemophilia-like condition. The significance of the test is not yet known. In the newly discovered hemophilia disease of swine the coagulation time of recalcified plasma is positive just as it is in human hemophilia, which this so closely resembles. Tonniquet tests were positive on several occasions and spontaneous nontraumatic hemorrhages occurred. The coagulation defect, however, appears to be primarily in plasma and the authors consider the term hemophiloid an appropriate one.

Differential Diagnosis of Hemophilia and Hemophilia-Like Diseases

	Human Hemophilia	Swine Hemophilia	Hemophilia Like Disease in Women
Hereditary	Recessive sex-linked	Simple recessive	
Sex	Male	Male & female	Female
Transmission	Female	Male & female	
Onset	Infancy	Early	Adulthood
Bleeding Type	Deep	Deep	Deep
Cause	Traumatic	Traumatic	Spontaneous
Coagulation	Delayed	Delayed	Delayed
Clotting time of recalcified plasma	Positive	Positive	Negative

HEMATURIA ASSOCIATED WITH HEMORRHAGIC DIATHESIS *R. Winston Evans and H. C. Mc Lerr* Lancet II 175 1945

The authors present the case and familial history of a patient with a hemorrhagic diathesis symptomatically resembling hemophilia.

Case Report. A 39 year old soldier of Greek birth suffered his fourth attack of hematuria in 3 months. The sequence of events was the same in each instance—in the course of his work he lifted a heavy weight and about 4 hours later passed bloody urine with considerable pain. The past history revealed that he had been severely incapacitated from birth to the age of 24, with skin, mucosal, visceral, muscle and even on one occasion cerebral hemorrhages and for the next 10 years bled profusely and for long periods from minor cuts and wounds. Examination revealed a large submucosal hemorrhage centered at the left ureteral orifice, hyperextensibility of the joints, rudimentary toe nails, and undue glossiness of the toenails. It was otherwise negative. In spite of the severe bleeding there was no anemia. Coagulation, prothrombin time and platelets were normal. Bleedings times done by the Duke and saline methods were normal but bleeding began again spontaneously about 5 minutes after the completion of each test and continued for a further two minutes.

Investigation indicated that the family suffered from similar and often fatal hemorrhagic tendencies affecting 37 of 61 males and females for at least 4 generations. The tendency seemed to be inherited as a Mendelian dominant and was inbred through marriage between cousins twice removed. Although the patient could not remember exactly, he suggested that rudimentary toe nails and hyperextensibility of the joints were associated with the tendency to bleed in his family history. The authors propose that this association may well be due to some hereditary anomaly of collagen condensation. The capillaries could not be examined for distortion and evidence of deficient power of contractility.

HYPOPROTHROMBINEMIC ACTION OF QUININE SULFATE *Leo A Pirk and Reeba Engelberg J A M A 128*
1093-95, 1945

The authors studied the effect of quinine sulfate on the prothrombin time of normal subjects. Five normal males ranging in ages from 18 to 43 years, whose nutritional state was satisfactory and whose prothrombin times were normal, were given single 5 grain doses of quinine sulfate by mouth daily for periods ranging from 6 to 16 days. In all cases the drug produced a significant increase in the prothrombin time, varying from 5 to 11.8 seconds. The times promptly regressed on discontinuation of the drug. On repeat testing, the concurrent administration of vitamin K afforded full protection in all individuals from this quinine sulfate produced hypoprothrombinemia. Neither the minimal hypoprothrombinemic dose of quinine nor the optimal vitamin K dose has been established yet.

The authors urge the prophylactic administration of vitamin K to troops receiving quinine sulfate for the purpose of eliminating the danger of prolonged bleeding from wounds.

PROTHROMBIN ACTIVITY IN RATS WITH HEPATIC AND OTHER TUMORS *John B Field, C A Baumann and A P Link Cancer Research 4 768 1944*

Various investigations indicate that the synthesis of prothrombin probably occurs in the liver. Rats with large primary hepatic tumors were used for the testing of prothrombin activity before and after the administration of dicoumarol. Simultaneous observations were made on normal rats and on those bearing tumors in other parts of the body. The rats with spontaneous mammary tumors, induced skin tumors or inoculated Flexner-Jobling tumors did not show a prolongation of the normal prothrombin time. On the other hand, the presence of primary hepatic tumors may cause a mild hypoprothrombinemia. Dicoumarol in standard doses (2.5 mg) usually caused a more severe hypoprothrombinemia in rats with primary hepatic tumors than in the other rats. The extent and duration of the hypoprothrombinemia is probably influenced by the amount of normal hepatic tissue present. Vitamin K protected normal rats from this dicoumarol effect, but the protective action is either lessened or abolished in those with primary hepatic tumors.

IDIOPATHIC HYPOPROTHROMBINEMIA WITH REPORT OF A CASE *V T Austin and H Quastler Am J M Sc*
210 491-500 1945

A case of hypoprothrombinemia is presented. The patient, a 56 year old white male, presented with massive hemorrhages into various muscles from mucous membranes, the gastro-intestinal and genito-urinary tracts, and a marked anemia, with remissions and exacerbations leading to death in a few months. Laboratory data revealed normal bleeding time, increased coagulation time, poor clot retraction, normal platelets and a negative tourniquet test. The patient was Rh negative but no evidence of an anti-Rh agglutinin was found. The prothrombin time was markedly prolonged, prothrombin concentration low, blood calcium and fibrinogen were normal. A sternal puncture revealed normal marrow. Autopsy revealed scattered hemorrhages and granuloma (questionably tuberculous) of the lungs, liver, and nodes. The possibility that this might be Hodgkin's disease or sarcoidosis was considered. However, the authors do not feel that there was a causal relationship between the hypoprothrombinemia and other conditions. Causes for secondary hypoprothrombinemia were ruled out. There was no evidence for K-avitaminosis and there was a lack of response to various vitamin K preparations. Autopsy showed minimal liver damage and liver function tests were negative after fully developed bleeding tendencies were established. Therefore there was no evidence for vitamin K fastness secondary to liver damage. There was no dicoumarol administration. The authors had previously reported 4 other cases of idiopathic (questioned) hypoprothrombinemia and realize that this case differs from the others in certain fundamental differences (i.e., age of onset, familial history, course, coagulation time, clot retraction and bleeding, and tourniquet tests) and conclude that cases thus termed represent more than one disease.

HYPOPROTHROMBINEMIA INDUCED IN SUCKLING RATS BY FEEDING THEIR MOTHERS HYDROXYCOUMARIN AND ACETYSALICYLIC ACID *John B Field Am J Physiol 143 238 1945*

Shortly after hydroxycoumarin was discovered as the causative agent of hemorrhagic sweet clover disease it was observed by Field, Overman, and Baumann that pregnant and lactating rats tolerate higher levels of the anticoagulant than normal females and in an extension of this study it was noted that continuous feedings of the anticoagulant to female rats with suckling pups caused hemorrhages to appear

in the young. The authors demonstrated that the drug fed lactating rats produced a hypoprothrombinemia in the sucklings and the suckling pups are subject to hemorrhages. It is not known whether the drug passes directly through the milk or whether a metabolite from it is the cause of the hypoprothrombinemia and hemorrhage in the young. Vitamin K afforded a greater protective action in the pup than in the mother but it cannot yet be stated whether the protective action is due to the transmission through the mammary gland of the intact vitamin K or an active metabolite. Hypoprothrombinemia can also be induced in suckling rats by giving large quantities of acetylsalicylic acid to the mothers. Davis and Porter in the *British Medical Journal* May 27, 1944, reported on the favorable clinical results of the treatment of puerperal thrombosis with dicoumarol.

It would appear that removing the suckling from the mother or administering vitamin K prophylactically to the baby would be advisable.

STUDIES ON THE HEMORRHAGIC SWEET CLOVER DISEASE John B. Field, Earl G. Larsen, Leonard Spertzel, and Karl B. Link. *J. Biol. Chem.* 156: 725, 1944.

The authors conducted experiments to show that the methylxanthines (caffeine, theobromine, and theophylline) induced in the dog, rat, and rabbit a state of hyperprothrombinemia as reflected by shortened plasma prothrombin times and indicate that, as a result of the induced hyperprothrombinemia, the hypoprothrombinemic action of the anticoagulant dicoumarol is lessened.

The hyperprothrombinemic effect was not exhibited by other purines, pyrimidines, and related compounds. The action from a single dose of methylxanthine persisted 4 to 5 days in the dog. Through repeated small doses, the action was maintained for periods up to 30 days. When the methylxanthines were given either with or 24 hours after the anticoagulant they not only reduced the intensity of the hypoprothrombinemic response but also shortened its duration. Single doses of the methylxanthines protect a standardized dog against repeated doses of dicoumarol for periods up to 14 weeks. Continued ingestion of caffeine and theobromine prolonged the survival time of rats fed the anticoagulant daily. The authors suggest that the methylxanthines provide a functional stimulation of hepatic tissue which accounts for the hyperprothrombinemia in normal animals and for the protective action against the anticoagulant. They caution that their prolonged use in cardiacs might augment the tendency for thrombus formation which is a frequent complication.

THE EFFECT OF PENICILLIN ON HEPARIN TOLERANCE L. E. Hines and D. L. Kessler. *J. A. M. A.* 138: 744, 1945.

The authors present 2 cases of proved bacterial endocarditis which showed early improvement following penicillin therapy but fatal termination from hemorrhage. One patient, a 37 year old female, was treated with penicillin only, with a total of 700,000 units and died with extensive cerebral hemorrhage. The other, a 22 year old white pregnant female, received 34 grams of sulfamerazine and 1 month later delivered a viable 7 months baby. At this time, a course of 1,200,000 units of penicillin and 1 Gm. of heparin was given. The patient died 1 month later. Autopsy revealed widespread intraperitoneal, mesenteric, and pleural hemorrhages. This prompted the authors to investigate the effect of penicillin on heparin tolerance. Studies of prothrombin times, platelet counts, and heparin tolerance curves were made on 10 cases before, during, and after penicillin treatment. Two patients showed a spectacular delay in coagulation time occurring after administration of 10 mgm. of heparin with a 2 to 7 fold increase over the normal levels; in 5 cases mild increases of 1 to 2 minutes were noted, and in 3 cases no changes occurred. No changes in the other determinations (platelets, prothrombin time, RBC, Hgb) were noted. The tendency to this change in the tolerance is important in the treatment of bacterial endocarditis if penicillin is used with adjuvant heparin therapy, and the authors suggest that it is advisable to run heparin tolerance tests as a precautionary measure when both drugs are used at the same time.

PENICILLIN EFFECTS ON BLOOD COAGULATIONS L. Moldovsky, W. B. Hasselbrock, C. Cateno, and D. Gordon. *Science* 102: 38, 1945.

Twenty normal subjects were administered penicillin by oral and intramuscular routes and its effect on blood coagulation was studied. After having first established base line determinations on the penicillin level, clotting, bleeding, and prothrombin times were made at 15 and 30 minute intervals after administration of the drug. The patients were found to show a marked fall in the clotting time which occurred

in inverse ratio to the penicillin concentration, and it persisted at a depressed level even after penicillin had completely disappeared from the blood, in some cases for as long as 1 hour. Bleeding times also fell, but the effects were not marked and were transient. Prothrombin times did not show a unidirectional change. A striking alteration in the blood as the penicillin levels rose was the production of a nonretractile clot.

The authors maintain that penicillin is conducive to thrombus formation and suggest investigative studies to test the value of the drug as a coagulant in hemorrhagic disorders. This paper contradicts the results obtained in the previous paper abstracted here, and further investigation is indicated to establish the effect of penicillin on coagulation.

STUDIES IN BLOOD COAGULATION (IN VITRO) *F H L Taylor, C S Davidson, H S Tagnon, M A Adams, A H MacDonald and G R Minor* *J Clin Investigation* 14: 698-704, 1945

The authors have prepared two protein fractions from cell and calcium free human plasma both of which have a definite effect in lowering the coagulation time of hemophilic blood. One of these protein fractions requires the presence of both calcium ion and prothrombin to exhibit its activity while the other acts as a true thrombin since it can convert fibrinogen to fibrin in the absence of calcium and prothrombin. Fibrinogen is the plasma protein coagulating on the addition of thrombin only. Prothrombin was detected by a modification of Quick's method using 0.1 cc of the plasma fractions rather than of whole plasma. Several fractions of plasma containing varying amounts of albumin, alpha, beta, and gamma globulin and fibrinogen were tested for antihemophilic activity. The greatest activity occurred with that fraction called Fraction I by the authors—which contained the largest amount of fibrinogen (61 per cent) and which also appears to contain the antihemophilic fraction. These investigators hope to subfractionate a highly potent injectable material from Fraction I for use in the treatment of hemophilia.

THE COAGULATION DEFECT IN HEMOPHILIA: The Effect in Hemophilia of the Parenteral Administration of a Fraction of the Plasma Globulin Rich in Fibrinogen and Anti hemophilic activity. *George Minor, C S Davidson, J H Lewis, H J Tagnon, F H L Taylor* *J Clin Investigation* 24: 704-08, 1945

Some preliminary observations on the in vivo effect of the administration of Fraction I of plasma to hemophiliacs are presented. This fraction contains 60-70 per cent fibrinogen together with smaller amounts of the other globulins. The intravenous or intramuscular administration of doses varying from 11.5 to more than 125 mg of Fraction I of pooled human plasma reduced the coagulation time of hemophilic blood toward or to normal values in 15 of 16 cases in which it was employed. Fibrinogen is not the active antihemophilic principle in this fraction. The dosage for therapeutic use has not yet been established. It was found that 200 to 600 mg of the globulin have an effect equal to that obtained by 80 cc of fresh plasma or 100 cc of fresh whole blood. The only untoward reaction observed was a slight sclerosis of the injected vein in one case and this was thought to be probably due to the high concentration of the injected material. There was no change in prothrombin times. Injections of this active globulin fraction had no influence on the effectiveness of subsequent injections of the material.

The work of these investigators suggests that soon a substance may be available whereby hemophiliacs may be maintained in a relatively normal state by the injection of maintenance doses of the effective plasma protein.

FURTHER OBSERVATIONS ON THE EFFECT OF BENZENE ON A STRAIN OF MYELOID CHLOROLEUKEMIA IN MICE AND ON CHANGES PRODUCED IN THE LEUKEMIC CELLS BY THE CHEMICAL*

Bj C M FLORY, M D , Ph D , I D STEINHARDT, M D , AND J FURTH, M D

NUMEROUS attempts to control mammalian leukemias have, in general, been unsuccessful ^{1,4} Previous studies² on the chemotherapy of transmitted mouse leukemias showed that benzene markedly retarded the development of the myeloid chloroleukemia 1394 when administered soon after the introduction of the leukemic cells, and that it prolonged the lives of animals to some extent when the treatment was begun after the disease was far advanced. In the present paper the effect of prolonged treatment with benzene on mice with this chloroleukemia is described, and evidence is presented that administration of this chemical in large amounts causes the destruction of many of the leukemic cells and results in a diminution in the size of spleens infiltrated with this leukemia, and of subcutaneous tumors composed of the leukemic cells.

The usefulness of transmitted mouse leukemia in the study of this disease has been discussed previously ^{1,2} Leukemia in mice is similar, in almost every respect, to the disease in man. Lymphoid, myeloid, and monocytic leukemias have been observed in both species, and the histologic appearances of the leukemic cells and of the organs infiltrated by these cells are similar. Leukemia in mice is generally considered to be a neoplastic disease. In certain inbred strains of mice the incidence of spontaneous leukemia exceeds 70 per cent. In such a strain of mice the disease can readily be passed from animal to animal by the subcutaneous implantation or intravenous injection of leukemic cells, and will kill almost 100 per cent of the animals. It is possible to inject fifty or a hundred mice with a single strain of leukemic cells, and then study the effects of one or several therapeutic agents on the transmitted disease in some of these animals, keeping others as controls.

MATERIALS AND METHODS

The mice used were either of the inbred Ak Stock or its first generation hybrids. All mice used in the first five experiments were of similar weight, and from 7 to 8 weeks of age. The mice used in experiment 6 were about 15 weeks of age.

The strain of transmissible myeloid chloroleukemia (1394) and the preparation of the suspensions of leukemic cells used for intravenous injection have been previously described.² The cells were injected into the tail veins of the mice. After all of the mice selected for a given experiment had been injected they were divided into comparable groups.

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The subcutaneous tumors were produced by implanting a small fragment of leukemic spleen or lymph node, previously rinsed in Tyrode's solution, into the subcutaneous tissues of the mice. A tumor formed at the site of the implant after about one week and grew steadily. The tumor tissue was then used for the next transfer. At first the mice died with generalized leukemia while the tumor was small, but after several transfers the tumors grew to 2 or more cm in diameter before the animals succumbed to generalized leukemia. Although the length of life of animals bearing subcutaneous tumors was longer and the period of survival of the tumor-bearing animals more variable than that of mice in which the leukemic cells were introduced intravenously, the establishment of subcutaneous tumors enabled direct observation on the effect of benzene on mice bearing these tumors.

The benzene was always given orally in cottonseed oil, the total volume of each feeding being $\frac{1}{10}$ cc. Feedings were given with a curved blunt needle.⁵ Unless otherwise stated, treatment was begun 24 hours after the introduction of the leukemic cells.

The weights of the animals were recorded twice weekly. In experiments 1 to 5 no instances of weight loss were observed, in experiment 6 the treated animals lost weight, as will be discussed subsequently.

All mice were autopsied, and whenever the diagnosis was in doubt sections were studied microscopically.

White blood cell counts and differential white blood cell counts were performed on some animals with this chloroleukemia. Leukemic cells were found regularly in the circulating blood when the disease was far advanced, though only in small numbers.

EXPERIMENTAL

Five milligrams of benzene given orally six times weekly in cottonseed oil are tolerated for many months by mice weighing over 20 Gm. Ten or 25 mg are toxic after a week or two.²

A Effect of prolonged administration of benzene on the myeloid chloroleukemia 1394

In experiment 1 (table 1) 5 mg of benzene were given six times weekly for from 167 days to almost one year. All of the untreated mice died of chloroleukemia, the average duration of life after the injection of the leukemic cells being 58 days. One of the mice treated with benzene died of leukemia on the 170th day, the other five were killed on the 362nd day. Three of these mice had not been given benzene for the last 195 days of the experiment. Microscopic examination of the liver, spleen, lymph nodes, and bone marrow of four of these mice failed to reveal evidence of leukemia. One mouse had an early lymphoid leukemia which was probably spontaneous, or which may have been induced by the benzene.

In experiment 2 (table 1) the mice were given benzene in the same manner as in experiment 1. The treated mice lived over three times as long as the untreated mice, though all eventually died of chloroleukemia. In the same experiment treat

ment by underfeeding* prolonged the average survival time by over one-third, and x-ray by over three-fourths

In experiment 3 (table 1) the benzene-treated mice were killed after they had survived over three times as long as the untreated animals. At autopsy, one treated

TABLE 1—Effect of Prolonged Treatment with Benzene¹ on the Myeloid Chloroleukemia 1394

Exp no	Treatment	No of mice injected	Mice dying of leukemia			Mice killed ²		
			No	Survival in days ³		No +	No -	No of days ⁴
				Extreme	Average			
1	None (controls)	6	6	31-72	58	0	5	362
	Benzene, 5 mg 6 times weekly	6	1	170	170			
	Radiophosphorus ⁵	5	5	56-88	68			
2	None (controls)	6	6	23-32	27			
	Benzene, 5 mg 6 times weekly	6	6	76-118	94			
	Underfeeding	6	6	34-44	39			
	X ray ⁶ 250 r on 2nd, 9th, 27th, 39th, and 50th days	5	5	34-73	51			
	Other chemicals ⁶	22	22	19-32	27			
3	None (controls)	6	6	33-44	36			
	Benzene, 5 mg 6 times weekly	6				1	5	122
	Other chemicals ⁶	40	40	34-52	42			
4	None (controls)	6	6	32-77	45 ⁷			
	Benzene, 5 mg 6 times weekly for 20 times	5	4	60-81	67	0	1	200
	for 40 times	4	1	109	109	0	3	200
	for 60 times	4	3	94-120	107	0	1	200
	continuous until death	5	4	63-159	105	0	1	200

¹ The benzene was given orally in cottonseed oil beginning the day after the injection of the leukemic cells and ending with the death of the animal in all experiments except no. 44 (see text)

² The symbol + indicates that the animals had leukemia when killed, - indicates that they apparently were free from the disease

³ The number of days refers to the days after the injection of the leukemic cells

⁴ The method of administration of radiophosphorus and its effects are described elsewhere¹

⁵ The mice to be irradiated were placed in a meshed wire cage and exposed to 140 kv, 5 ma x ray at 30 cm target skin distance with no filter at a rate of 60 r per minute

⁶ The negative results with these chemicals will be reported in a list of compounds which have been tested for chemotherapeutic action on cancer which is being compiled by Dr Dyer²

⁷ One animal survived the rest of the group by 36 days dying on the 77th day from chloroleukemia. Without it the average survival time would be shorter

mouse had moderately advanced chloroleukemia, the other 5 appeared normal on macroscopic examination, and the organs of 2 of these were examined histologically and no leukemia was found

* The method of underfeeding has been previously described²

Experiment 4 (table 1) shows the effect of varying the length of treatment with benzene. In the group of mice which received 20 doses of benzene the average survival time was prolonged by about one-half, while in the mice which received 40 or more treatments the duration of life was more than doubled. One or more mice of each group of the benzene-treated animals, a total of 6 mice, survived until the 200th day, when they were killed, and microscopic examination of the livers, spleens, and bone marrow of these animals failed to reveal evidence of leukemia.

B Effect of treatment with benzene on the sizes of the spleens of mice with the myeloid chloroleukemia 1394

In an experiment previously reported,² treatment with benzene prolonged the lives of mice with the chloroleukemia even when treatment was begun after the disease was far advanced.

Mice dying of this strain of leukemia had massive infiltrations of the spleen by leukemic cells, and after death from this disease the size of the organ exceeded $19 \times 6 \times 4$ mm. The liver, bone marrow, and to a lesser extent the lymph nodes were similarly infiltrated. Since in the mouse even a slight enlargement of the spleen is palpable through the abdominal wall, changes in the size of the spleen can be closely followed.

The effect of benzene on the size of the spleen and on the survival of mice with advanced chloroleukemia was studied in experiment 5, and the results are shown in figure 1. Additional mice, killed for microscopic examination, are not included in the figure.

The sizes of the spleens of the 10 untreated mice, shown in figure 1, increased rapidly after the 20th day. The first mouse died on the 27th day and the last on the 33rd day, while the average survival time was about 30 days. At autopsy all mice showed advanced chloroleukemia, and the spleens were all greatly enlarged and varied from 19 to 26 mm. in length and 6 to 9 mm. in width, the thickness of most spleens was about 4 mm.

When 5 mg. of benzene was given six times weekly orally in cottonseed oil, beginning the day after the inoculation of the leukemic cells, the spleens increased in size at a much slower rate. The first mouse died on the 58th day, and the treatment was stopped on the 84th day. The last mouse died on the 102nd day, and the average survival time was about 80 days, or over twice as long as that of the untreated animals. At autopsy the spleens of the treated mice were greatly enlarged and of about the same size as those of the untreated animals. The livers and lymph nodes of all mice showed advanced chloroleukemia.

A third group of animals was not treated until late in the disease. Two of these mice died of advanced leukemia on the 24th day and another on the 25th day. On the 25th day the spleens of all mice were moderately or greatly enlarged, and treatment was begun with 5 mg. of benzene given 6 times weekly and was continued until the last mouse died. After treatment was instituted the rate of increase of the size of the spleens of most animals was reduced. In 6 mice the spleens did not increase further in size until after the 31st day, while the spleens of 3 became

larger and 2 smaller. One mouse died of advanced leukemia 3 hours after the first feeding of benzene, the next on the 32nd day, and the last on the 65th day. If the mouse which died 3 hours after treatment is included, the average survival time is about 44 days, otherwise, as listed in figure 1, about 46 days. At the time of death all mice had advanced chloroleukemia.

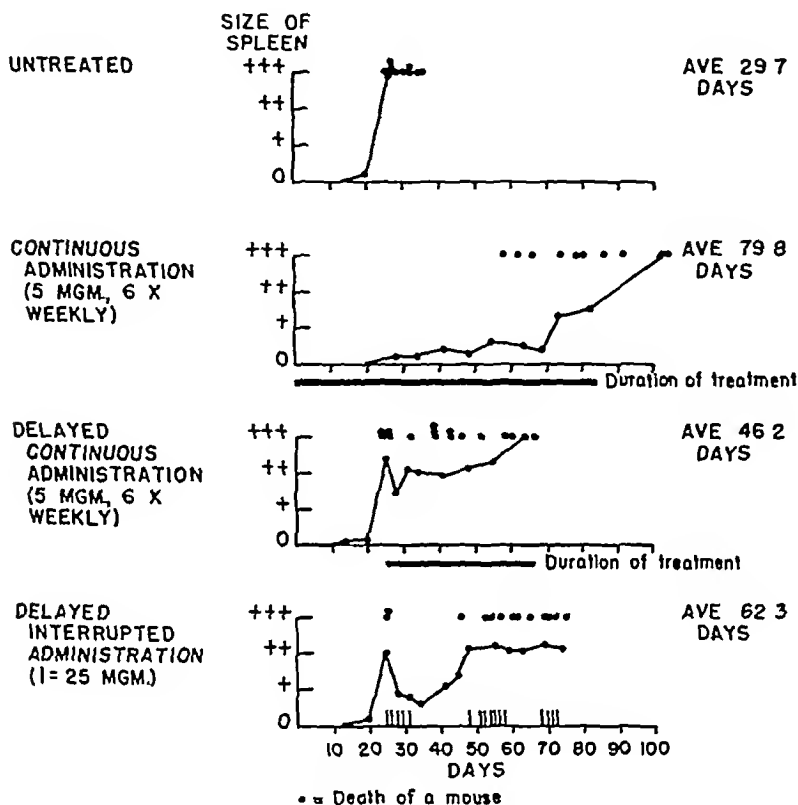


FIG. 1. THE EFFECT OF THE ADMINISTRATION OF BENZENE ON THE AVERAGE SIZE OF THE SPLEENS OF MICE WITH THE MYELOID CHLOROLEUKEMIA 1394

The benzene was given orally in cottonseed oil. The size of the spleen was determined by palpation. The symbol + indicates that the spleen was slightly enlarged as determined by palpation, ++ indicates moderate, and +++ great enlargement of the spleen, the organ measuring at least $18 \times 7 \times 4$ mm.

In the fourth group one mouse died of leukemia on the 25th day. On the same day one of the remaining mice that was given 50 mg of benzene died 90 minutes after the treatment, because of this the remaining 11 animals were given only 25 mg of benzene on the 25th, 26th, 27th, 28th, and 30th days. This caused the spleens to decrease in size. Before the administration of the chemical, the spleen of one mouse was greatly enlarged, those of 10 were moderately enlarged, that of one slightly enlarged, while one had no enlargement of the organ. On the 34th day one mouse had a moderately enlarged spleen, 4 had slightly enlarged spleens, and the remaining 6 had no palpable enlargement. After the spleens had again

increased in size, a second course of treatment was instituted, but this had little effect on the size of the spleens. The first of the 11 mice died on the 47th day, the last on the 75th day, the average survival period for 11 mice was about 62 days, which was more than twice as long as the average survival period in the untreated animals. At death the spleens were greatly enlarged and similar to those of the untreated animals, on microscopic examination the spleens and livers, and the lymph nodes as well, showed advanced chloroleukemia.

TABLE 2 (Experiment 6)—Effects of Treatment with Benzene on the Sizes of Subcutaneous Tumors Composed of Cells of the Myeloid Chloroleukemia 1394¹

No of mice untreated	Days ² after implantation of tumor									Day of recurrence of tumor	Day of death	Autopsy	
	9	15	16	20	25	29	36	44	50			Size of tumor mm	Generalized leukemia
1	2 x 2	3 x 5	5 x 5	5 x 8	8 x 10	9 x 13	11 x 21				39	11 x 20	present
2	1 x 4	3 x 5	4 x 6	5 x 7	5 x 12	8 x 19	11 x 21				36	11 x 21	present
3	3 x 3	5 x 7	6 x 8	7 x 10	7 x 12	9 x 15	10 x 16				35	10 x 16	present
4	2 x 3	6 x 7	8 x 8	7 x 11	9 x 13	10 x 16	11 x 16				36	11 x 16	present
5	3 x 3	3 x 5	4 x 5	4 x 6	3 x 6	5 x 6	6 x 7	8 x 9	8 x 10		109	14 x 19	present
6	2 x 2	4 x 6	4 x 6	5 x 8	7 x 10	7 x 11	9 x 11	13 x 16			46	15 x 17	present
7	4 x 4	4 x 5	4 x 4	4 x 5	5 x 5	5 x 7	7 x 10	12 x 14			63	22 x 29	present
8	2 x 3	5 x 6	6 x 7	8 x 10	13 x 16	13 x 16	14 x 16				37	13 x 20	present
↑ Start of treatment													
No of mice treated with benzene													
9	2 x 5	4 x 7	4 x 6	2 x 3	1 x 2	1 x 1	0	0	0		174	0	present
10	2 x 2	7 x 8	7 x 7	4 x 4	3 x 4	0	0	0	0	72	81	7 x 7	present
11	3 x 4	5 x 7	6 x 7	3 x 5	0	0	0	0	0	72	97	8 x 15	present
12	3 x 3	3 x 6	5 x 6	1 x 2	1 x 3	1 x 1	0	0	0	0	240*	0	absent
13	2 x 3	4 x 6	4 x 6	2 x 3	1 x 1	0	0	0	0	116	166	11 x 22	present
14	3 x 3	4 x 5	4 x 4	3 x 3	2 x 2	1 x 2	1 x 1	0	0	0	240*	0	absent
15	3 x 3	4 x 6	5 x 6	1 x 2	1 x 1	1 x 1	0	0	0	85	110	1 x 2	present
16	4 x 5	7 x 8	7 x 7	3 x 4	2 x 3	0	0	0	5 x 7	50	78	12 x 20	present

* Killed

¹ In this table the length and the width of the tumors are expressed in mm. The tumors were measured more frequently than is indicated by the table and the record from 50 days on is expressed only by noting the day of recurrence of the tumor. Once the tumors recurred they grew steadily.

² 'Days' refers to the days after the implantation of the tumor particles.

Gross and microscopic examinations of unselected mice of the first two groups were made at intervals. The mice killed for this purpose are not included in figure 1.

In the untreated group one mouse was killed on the 6th and others on the 13th, the 20th, and the 27th days. In the mouse killed on the 6th day no leukemic cells were seen in sections of liver and spleen, but in the mouse killed on the 13th day many small scattered foci of leukemic cells were present in sections of both organs. In the animal killed on the 20th day (figure 2) the leukemic cells were more abundant in these organs. In the mouse killed on the 27th day about one-third of the liver and spleen was composed of leukemic cells, and this was true also in an animal that died on the 32nd day (figure 3).

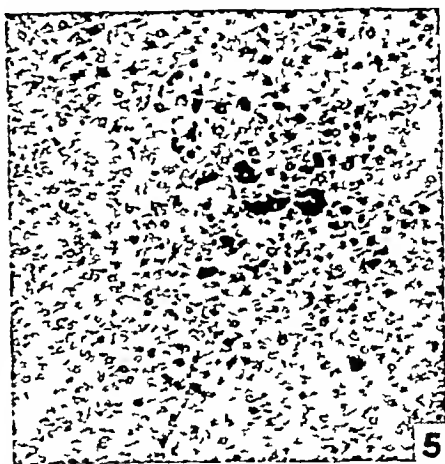
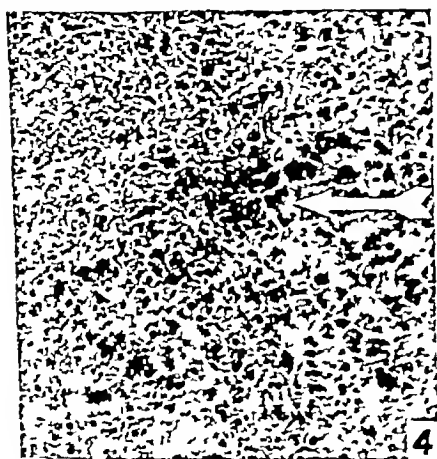
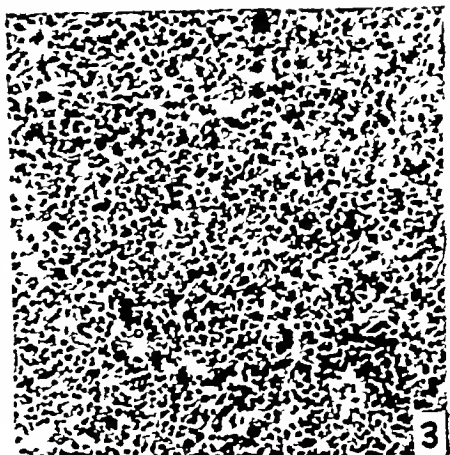
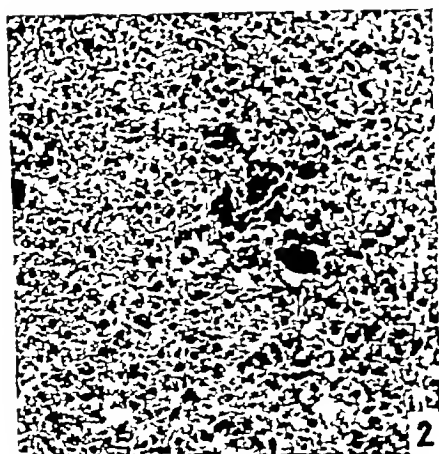


FIG 2 Early leukemic infiltration in the liver of a mouse injected intravenously with the cells of the myeloid chloroleukemia 1394 and killed 20 days later. No treatment was given. The scattered foci of hyperchromatic leukemic cells constitute but little of the bulk of the liver (180X).

FIG 3 Massive leukemic infiltration of the liver of an untreated mouse which died on the 32nd day after the intravenous injection of the leukemic cells. About half of the liver is composed of these cells (180X).

FIG 4 Early leukemic infiltration in the liver of a mouse injected intravenously with the cells of the myeloid chloroleukemia 1394 and killed 34 days later. This animal was treated with 5 mg of benzene 6 times weekly. The arrow points to a focus of small leukemic cells. At this time all untreated mice had died of leukemia (180X).

FIG 5 Early leukemic infiltration in the liver of a mouse injected intravenously with the cells of the chloroleukemia and killed 48 days later. This animal was also treated with 5 mg of benzene 6 times weekly. The degree of leukemic infiltration is similar to that seen in untreated mice 20 days after the injection of the leukemic cells (fig. 1) (180X).

In the group of mice treated with 5 mg of benzene beginning on the 2nd day, one mouse was killed on the 6th day and one each on the 20th, the 34th, and on the 48th days. No leukemic cells could be seen in the sections of the livers or spleens of the animals killed before the 34th day. The liver of the animal killed

on the 34th day contained small foci of leukemic cells (figure 4), and that of the mouse killed on the 48th day had a few scattered larger foci of leukemic cells (figure 5). Treatment with benzene apparently retarded the leukemic infiltration of the livers and spleens.

C The effect of the administration of benzene on the sizes of subcutaneous leukemic tumors composed of the cells of the myeloid chloroleukemia 1394

In table 2 (experiment 6) is shown the effect of the administration of benzene on subcutaneous tumors composed of the cells of the chloroleukemia.

Sixteen mice carrying small subcutaneous tumors as the result of the implantation of small particles of tumor 9 days before were divided into two equal groups. On the 15th day after the implantation, one group was treated with benzene, the other serving as controls. The treated mice were given 25 mg of benzene orally in cottonseed oil on the 15th, 16th, 18th, 19th, and 20th days after the implantation of the tumor particles, then 5 mg six times weekly until the 126th day of the experiment, when treatment was stopped. After the 3rd treatment with benzene, the tumors of treated mice became smaller. The first tumor disappeared on the 8th day of benzene therapy, and by the 23rd day of treatment all of the tumors of the treated mice had disappeared. Since then the tumors in 5 animals have recurred at the sites of the inoculation, and these animals have died of generalized chloroleukemia, as has also a 6th mouse which had no local recurrence of the tumor. Two mice were killed on the 240th day after the implantation of the tumor. No tumor was visible at the site of the original tumor mass, and microscopic examination of the liver, spleen, and lymph nodes failed to reveal evidence of leukemia.

The treated mice lost some weight, and on the 22nd day their average weight was only 20.1 Gm (range 14 to 28 Gm) as compared with an average weight of the untreated mice of 26.1 Gm (range of 23 to 30 Gm), but subsequently, while on the smaller dose of benzene, the treated mice gradually gained weight. The tumors of 4 underfed mice that were not given benzene did not decrease in size, although the weights of 2 of these mice dropped to 14 and 16 Gm, as compared with 19 to 25 Gm for those fed a normal diet.

Microscopic examinations were made of subcutaneous tumors under 1 cm in diameter in 7 untreated mice and in a similar number of animals treated with benzene. The tumors of the untreated mice were of the same size as those of the treated animals, and their hosts were killed at the same time.

In all tumors of the untreated mice there were small or large central areas of necrosis, but in the remainder of the tumor the structure of the cells was well preserved and only a few cells had pyknotic or fragmented nuclei (figure 6).

In the tumor of a mouse which had been given 100 mg of benzene in divided doses over a period of 2 hours and was killed 2 hours after the last dose, about one-tenth of the nuclei of the tumor cells were deeply pyknotic (figure 7). In 2 animals, killed 24 hours after a single oral dose of 25 mg of benzene in cottonseed oil, the nuclei of many tumor cells were pyknotic or fragmented. In one tumor about one-fifth, and in the other about one-half of the cells (figure 8) were necrotic.

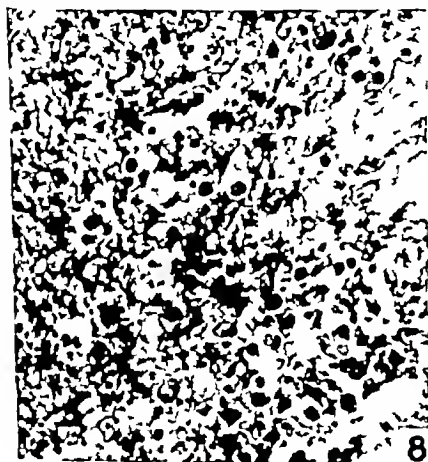
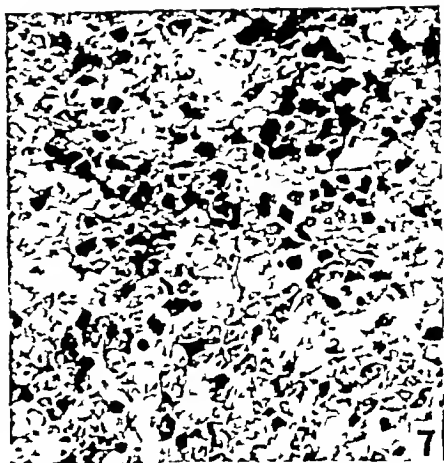
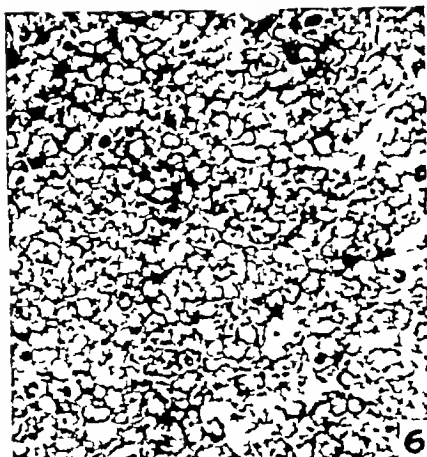


FIG 6 SUBCUTANEOUS TUMOR COMPOSED OF THE CELLS OF THE CHLOROLEUKEMIA

The mouse bearing this tumor was not treated (480X)

FIG 7 Early degenerative changes in a subcutaneous tumor composed of the cells of the chloroleukemia. The mouse bearing this tumor was given 100 mg of benzene orally in divided doses during a 2 hour period and was killed 2 hours after the last dose. About one tenth of the tumor cell nuclei are deeply pyknotic (480X)

FIG 8 Moderate degenerative changes in a subcutaneous tumor composed of the cells of the chloroleukemia. The mouse bearing this tumor was given 25 mg of benzene orally and killed after 24 hours. About half of the tumor cells show extreme pyknosis or fragmentation of their nuclei (480X)

FIG 9 Advanced degenerative changes in a subcutaneous tumor composed of the cells of the chloroleukemia. The mouse bearing this tumor was given 25 mg of benzene on each of 2 days and then killed on the 3rd day. About four fifths of all tumor cells were necrotic. The black fragmented or clumped material is nuclear debris (480X)

In the tumors of 2 animals that had been given 25 mg of benzene on each of 2 days, and then killed on the 3rd day, about four-fifths of all tumor cells were necrotic (figure 9). There were no changes in the blood vessels and in nonleukemic cells in and about the tumor. There was, however, some atrophy of the bone marrow

In 2 animals killed after 3 days of treatment with a total of 75 mg of benzene and in one animal killed after 4 days of treatment with a total of 100 mg of benzene, degenerative changes in the tumor cells were marked and the bone marrow showed advanced atrophy of all blood-forming elements

DISCUSSION

In the experiments previously reported² treatment with benzene prolonged the survival time of mice with early and advanced chloroleukemia. In the present experiments this prolongation of the survival time by treatment with benzene was observed constantly. In an effort to learn more of the details of this phenomenon the effects of this treatment on the sizes of the spleens of leukemic mice were studied. Benzene in large doses (25 mg daily) given to leukemic mice with enlarged spleens caused a decrease in the size of the spleens. Subsequently the spleens became large again and failed to respond to additional treatment with the chemical. Treatment with smaller doses (5 mg daily) only seemed to slow the growth of the leukemic spleens. With continuous administration of this dose of benzene beginning the day after the inoculation of the cells the spleens increased in size very slowly as compared with those of the untreated mice. Microscopic study of the organs of the untreated mice and those treated early in the disease with 5 mg of benzene showed that the malignant cells appeared in the organs of the treated animals at a much later time than in those of the untreated mice, and that at corresponding periods of time the leukemic infiltrations of the livers and spleens of the untreated leukemic mice were much greater than those of the mice treated with benzene.

In experiment 6 (table 2) it was found that treatment of mice with large doses (25 mg) of benzene caused subcutaneous tumors composed of the cells of the chloroleukemia to disappear to palpation, though later they recurred in most instances and killed the animals. Microscopic examination of tumors from mice treated with this chemical showed massive destruction of the leukemic cells.

The evidence presented indicates that the cells of this chloroleukemia are sensitive to the treatment of their hosts with benzene, as indicated by prolongation of life of treated animals, whether in the early or late stage of the disease, by the diminution in the size of leukemic spleens, by the temporary disappearance of subcutaneous tumors composed of these cells, and by the microscopic evidence of damage to the leukemic cells by such treatment. Administration of small doses of benzene (5 mg daily) either retards the growth of leukemic cells, or speeds up the rate of their destruction, or does both. Larger doses seem to cause actual destruction of some of the leukemic cells.

In our previous experiments* all mice injected with the cells of the chloroleukemia and treated with benzene eventually died of the disease.² In the present experiments certain treated animals failed to manifest the disease. In experiments 1, 2, 3, and 4 (table 1) and experiment 5 (figure 1) all of the untreated mice, a total of 34, died of the chloroleukemia, and the entire group of 78 mice treated

* At the time of the publication of the previous report² 2 mice in experiment 22 and 3 mice in experiment 23 (table 5) were alive. All of these animals subsequently died with myeloid chloroleukemia 1331.

with agents other than benzene also died of the disease. Sixteen of the 46 mice treated with 5 mg of benzene beginning the day after the injection of the leukemic cells failed to manifest the disease.

Several explanations may be given for this failure of certain of the benzene-treated mice to die of the chloroleukemia. One possibility is that the treatment with benzene was begun so soon after the injection of the leukemic cells that the malignant cells did not have an opportunity to multiply and become adapted to their new hosts before their exposure to the chemical. It is also possible that the disease was still latent in some of these animals, although microscopic examination of the liver and spleens of 13 out of 16 mice failed to reveal evidence of chloroleukemia. In all experiments in which the treatment with benzene was begun after the generalized chloroleukemia was firmly established, as in experiments 5 and 6 of this paper and experiment 23,² no mice failed to die of the leukemia although the treatment was often of long duration. In such experiments, the cells probably were multiplying actively when treatment was begun.

The above theories do not, however, explain the failure of 2 animals, which previous to treatment were bearing subcutaneous leukemic tumors, to die of the disease. In these animals the tumors were actively growing when the treatment with benzene was begun, and they disappeared under treatment. Almost 7 months after the tumors were last observed the animals were killed and no evidence of local tumor or generalized leukemia found. Since no benzene was given during the last 114 days of the lives of these animals, it is possible that if latent leukemic cells existed, they would have grown by the time the animals were killed.

The mode of action of benzene on normal and leukemic cells is poorly understood. The problem has been discussed previously.^{1,2} Evidence is presented in this paper, however, that the chemical can destroy the cells of this chloroleukemia in the body, as evidenced by its effect in reducing the sizes of subcutaneous tumors composed of the cells of the chloroleukemia and of spleens infiltrated with the cells, and by degenerative changes seen in the chloroleukemic tumors after its administration.

The recurrence of most of the subcutaneous tumors composed of the cells of the chloroleukemia, the eventual increase in size of the leukemic spleens, and the ultimate deaths of most of these animals from generalized chloroleukemia indicate that the effect of benzene in most animals is only temporary. Recent work by Schwartz and Robbins⁶ suggests that the cells may acquire actual resistance to benzene.

The problem of the specificity of the action of benzene on this chloroleukemia requires clarification. It would be of interest to know if the cells of this chloroleukemia are more sensitive to benzene than the cells of the bone marrow and other organs. Since a dose of 5 mg of benzene may be given to a mouse six times weekly for almost a year without serious ill effect, yet will greatly prolong the lives of mice with chloroleukemia, it is probable that these leukemic cells are slightly more sensitive to the action of benzene than the bulk of the cells of the bone marrow and other vital organs. When larger doses of benzene are given atrophy of the bone marrow is produced, and although leukemic tissue actually

is destroyed by such doses, this amount of benzene will kill the animal if given for long periods of time

It is important to emphasize that in our experience most other mouse leukemias are not as sensitive to benzene as is the myeloid chloroleukemia 1394. This chloroleukemia is, out of a total of 3 myeloid and 4 lymphoid leukemias so far tested,^{1,2} by far the most sensitive to benzene. Certain transmitted mouse leukemias seem to be entirely refractory to the chemical. What the basis is of this exceptional susceptibility of these chloroleukemic cells to benzene remains to be determined.

SUMMARY

Oral administration of 5 mg of benzene six times weekly to 46 mice that had been injected 24 hours before with the cells of the myeloid chloroleukemia 1394, considerably prolonged the survival of the animals, and in 16 of these mice it seemed to prevent the development of the disease. The spleens of the animals so treated increased in size at a much slower rate than those of the untreated animals, and the leukemic infiltration of the organs was delayed.

Five mg of benzene given in a similar manner to mice with advanced chloroleukemia likewise prolonged the survival time of the mice and also slowed the rate of increase of the size of the spleens, 25 mg given five times in a 6 day period to mice with this leukemia brought about a marked reduction in the size of the spleens. Further treatments slowed the rate of enlargement of the spleens and survival was prolonged.

In 8 mice bearing subcutaneous tumors composed of the cells of the chloroleukemia the oral administration of 25 mg of benzene five times in a 6 day period followed by 5 mg given six times weekly brought about the disappearance of the local tumors as determined by palpation, but in 6 mice the tumors recurred and killed the animals. Microscopic examination of tumors of mice treated with benzene showed advanced degenerative changes in leukemic cells.

The authors wish to thank Miss Pauline Pope, Miss Mary Boon, and Miss Lucille Wolf for their technical assistance.

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CELLULAR CHANGES PRODUCED BY EXTRACTS OF HUMAN ORGANS

By L A ERF, M D , D L TURNER, PH D , AND F R MILLER, M D

THIS paper describes cellular infiltrations found in the organs of guinea-pigs that were injected subcutaneously with extracts made from organs obtained from patients dead of leukemia, Hodgkin's disease, and other diseases

The various organs were extracted by methods based on those of Turner and Miller,¹ who have produced cellular changes in the organs of guinea-pigs by injecting extracts of the urine and feces of patients with leukemia They have described three types of cellular reactions produced by these extracts and designated these changes as myeloid, lymphoid, and Hodgkin's types² These changes are produced by a keto-acid and a hydroxy-acid present in the extracts, and by mixtures of these two substances, respectively

MATERIALS AND METHODS

The patients had been dead from 1 to 19 hours, the cause of death was verified by clinical and laboratory data, and also by the findings at the necropsy The organs were ground with a meat chopper, the ground material was mixed with five times its weight of commercial methanol The mixture was acidified to Congo paper by the addition of hydrochloric acid and allowed to stand for 3 days, it was then filtered The filtrate was partially concentrated *in vacuo*, diluted with water, and extracted with petroleum ether (b p 30°-60°) The petroleum ether was washed with water and then with 2N sodium hydroxide solution The petroleum ether was then discarded, and the alkaline extracts were acidified and extracted with petroleum ether The ethereal solution containing acid and phenolic material was evaporated The residue was dissolved in four times its weight of acetone and cooled to -15° C The acids that crystallized were filtered and discarded, the mother liquor was evaporated In most cases the product was used in this form, it was usually a brownish-yellow, oily substance

In one case (Mont liver) the procedure was varied in the following manner The minced liver was boiled for 4 hours in a solution of 600 Gm sodium hydroxide in 4 liters of methanol The solution was diluted with water, acidified to Congo paper, and extracted with petroleum ether The petroleum ether solution was washed with water and evaporated The residue from the petroleum ether was separated into lead soaps, soluble and insoluble in ether, following the well-known methods of the literature^{3,4} The free acids obtained from the decomposition of the lead soaps insoluble in ether were purified further by crystallization at -15° C, in acetone as described above

The 28 guinea-pigs varied in weight from 350 to 450 grams They were all obtained from the same source After death or after sacrificing the animal—usually

From the Charlotte Drake Cardenza Foundation, Jefferson Medical College and Hospital, Philadelphia Pa

TABLE 1.—The Type and Degree of Cellular Infiltrations Found in Organs of Guinea pigs Subcutaneously Injected with Extracts of Leukemic Organs of Human Beings

Patient & disease or cause of death	Human organs from which extracts were made and weight	Total amt of extract given and weight of organ represented by extract	Dates of first and last injection of extract	Weeks between first injection and death	Date of death (K = killed, D = died)	Autopsy findings (at least 8 tissues of organs of guinea pig were studied, but only essential findings in relation to specific cellular infiltrations are recorded) Each guinea pig weighed between 350 and 450 Gm at start of experiment	Degree of cellular infiltration (L = lymphoid, M = myeloid, X = slight, XX = moderate, XXX = marked)
Camp, myeloid leukemia	Spleen (1650)	1 5 cc 250 Gm	1/22 to 3/2	7	K 3/17	#275 Liver, adrenals, and kidney—infiltrated with myeloid cells Spleen—many normoblasts Lymph nodes and marrow—hyperplastic	M X
		1 5 cc 250 Gm	1/22 to 3/2	6	D 3/3	#276 Liver, adrenals, and kidneys—infiltrated with myeloid cells	M X
	Liver (1500)	2 6 cc 260 Gm	2/23 to 4/6	6	K 4/6	#307 Kidney and liver—infiltrated with myeloid cells	M X
		1 5 cc 150 Gm	2/23 to 3/23	5	K 3/31	#308 Same as 307	M X
Mont, myeloid leukemia	Liver (1840)	2 1 cc 210 Gm	2/23 to 3/31	6	K 4/1	#304 Spleen very large (3 5 Gm) and had extramedullary hematopoiesis Liver and adrenals—infiltrated with myeloid cells	M XXX
		3 0 cc 310 Gm	2/23 to 4/13	7	K 4/16	#305 Kidneys, adrenals, and liver—infiltrated with myeloid cells	M XX
		2 3 cc 230 Gm	3/17 to 4/13	4	K 4/14	#346 Same as 305	M X
Brow, lymphoid leukemia	Spleen (1810)	1 6 cc 850 Gm	1/22 to 3/2	7	D 3/22	#281 Spleen (5 5 Gm) and lymph nodes—hyperplastic Liver—diffuse infiltration with lymphoid cells	L XXX
		1 6 cc 850 Gm	1/22 to 3/2	9	K 3/23	#282 Same as 281 Also marked increase in the lymphoid tissue of the submucosa of the G I tract	L XX

	Lymph nodes (700)	10 cc 300 Gm 10 cc 300 Gm	1/22 to 2/23 1/22 to 2/23	6 9	K 3/4 K 3/26	#283 Same as 281 #284 Similar to 281	L XX L X
Hoff, lymphoid leukemia	Spleen (600) & liver (3110) mixed	13 cc 290 Gm	1/22 to 2/23	8	K 3/18	#285 Kidneys, liver, and adrenals—infiltrated with lymphoid cells Lymph nodes—hyperplastic	L X
		13 cc 290 Gm	1/22 to 2/23	8	K 3/16	#286 Same as 285	L X
		16 cc 350 Gm	5/18 to 6/8	3	K 6/10	#419 Liver—marked infiltration	L XX
		17 cc 370 Gm	5/18 to 6/15	5	K 6/21	#420 Liver and kidney—focal areas of lymphoid cells Lymph nodes large	L XX
Prie, lymphoid leukemia	Liver (1880)	18 cc 800 Gm	4/20 to 5/5	4	K 5/18	#381 Liver, kidney, and adrenals—infiltrated with lymphoid cells	L X
		15 cc 650 Gm	5/5 to 5/18	8	K 7/9	#396 Same as 381 Lymph nodes—hyperplastic	L X

TABLE 2.—The Type and Degree of Cellular Infiltrations Found in Organs of Guinea pigs Subcutaneously Injected with Extracts of Organs of Human Beings Dead of Hodgkin's Disease, Heart Disease, or Cerebral Tumor

Patient & disease or cause of death	Human organs from which extracts were made & weight Gm	Total amt of extract given and weight of organ represented by extract	Dates of first and last injection of extract	Weeks between first injection and death	Date of death (K = killed, D = died)	Autopsy findings (8 tissues of organs of each guinea pig were studied. The essential findings are recorded)	Degree of cellular infiltration (Mo = monocytoïd, H = Hodgkin & like disease, — = very slight, X = slight, XX = moderate, XXX = marked)
Mitch, Hodgkin's disease	Liver (1240)	2.8 cc 600 Gm	2/14 to 3/10	5	K 3/19	#698 Liver slightly and diffusely infiltrated with polymorphonuclear and monocytoïd cells Spleen fibrotic Lymph nodes had areas of monocytes	H X
	Lymph nodes (1400)	2.8 cc 600 Gm	2/14 to 3/10	5	D 3/19	#699 Some lymph nodes had eosinophiles, monocytes, fibrosis, & giant cells resembling Hodgkin's disease	H XX
		2.0 cc 700 Gm	2/14 to 3/6	4	K 3/12	#700 Kidneys, adrenals, and lungs infiltrated with monocytes Occasional lymph node contained large areas of monocytes	H X
		2.0 cc 700 Gm	2/14 to 3/6	4	K 3/12	#701 Similar to 700	H X
Haue, heart disease (failure)	Liver (1570)	1.1 cc 130 Gm 1.1 cc 130 Gm	4/6 to 4/20 4/6 to 4/20	5 12	D 5/14 K 7/2	#368 Tissues normal Very slight infiltration of monocytes in adrenals #369 Similar to #368	Mo — X
	Liver (1380)	2.0 cc 550 Gm	4/20 to 5/18	4	K 5/18	#383 Tissues normal except for slight infiltration of lymphocytes in liver	L — X
Sill, cerebral tumor	Liver (1930)	1.6 cc 800 Gm 1.5 cc 700 Gm	5/25 to 6/8 5/25 to 6/8	5 8	K 7/1 K 7/22	#421 Tissues normal #422 Occasional small focal area of monocytes in liver and adrenal	Mo — X
	Liver (1670)	0.7 cc 570 Gm 0.7 cc 570 Gm	5/25 to 6/1 5/25 to 6/1	8 4	K 7/22 K 6/30	#423 Liver—focal areas of monocytoïd cells #424 Same as 423	Mo — X Mo — X

1 or 2 months after the initial injection—at least eight different organs were routinely sectioned and stained by the usual hematoxylin-eosin technic

RESULTS AND DISCUSSION

The experiments are summarized in tables 1 and 2. Table 1 indicates the type and degree of cellular infiltrations observed in the tissues of the guinea-pigs that

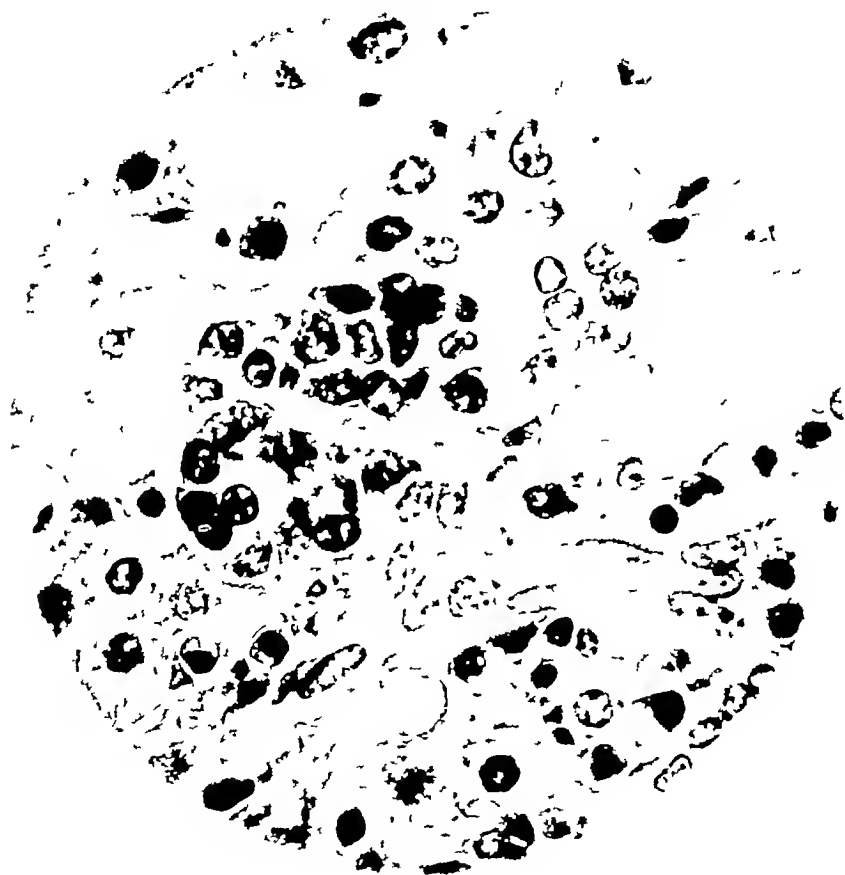


FIG. 1. MYELOID REACTION

Infiltration of liver of guinea pig with myeloid cells following injection of lipoid extract of human myeloid leukemic tissues

had received extracts of organs from patients who died of leukemia. The *myeloid reactions* were obtained from the tissues of 2 patients dead of myeloid leukemia, while the *lymphoid reactions* were obtained from 3 cases of lymphoid leukemia. These reactions were less extensive but similar to those found after injections of extracts of large quantities of urine of patients with leukemia,² indicating that the tissue extracts contained less of the keto-acid and hydroxy-acid than most of the urinary extracts.¹ The *myeloid reaction* occurred in varying degrees in all of the 7 guinea-pigs injected with extracts of myeloid leukemic tissues, and consisted of

- 1 infiltration or proliferation of immature red cells and myeloid cells, even occasionally megakaryocytes, between the liver cords and in the periportal spaces of the liver (see fig 1),
2. similar extramedullary hematopoiesis in the cortex and medulla of the adrenal,
- 3 myeloid proliferation or infiltration in the spleen, hypoplasia of lymph follicles,
- 4 occasional areas of myeloid proliferation in the kidney, between the tubules,
- 5 and evidence of hyperplasia of all of the elements of the bone marrow

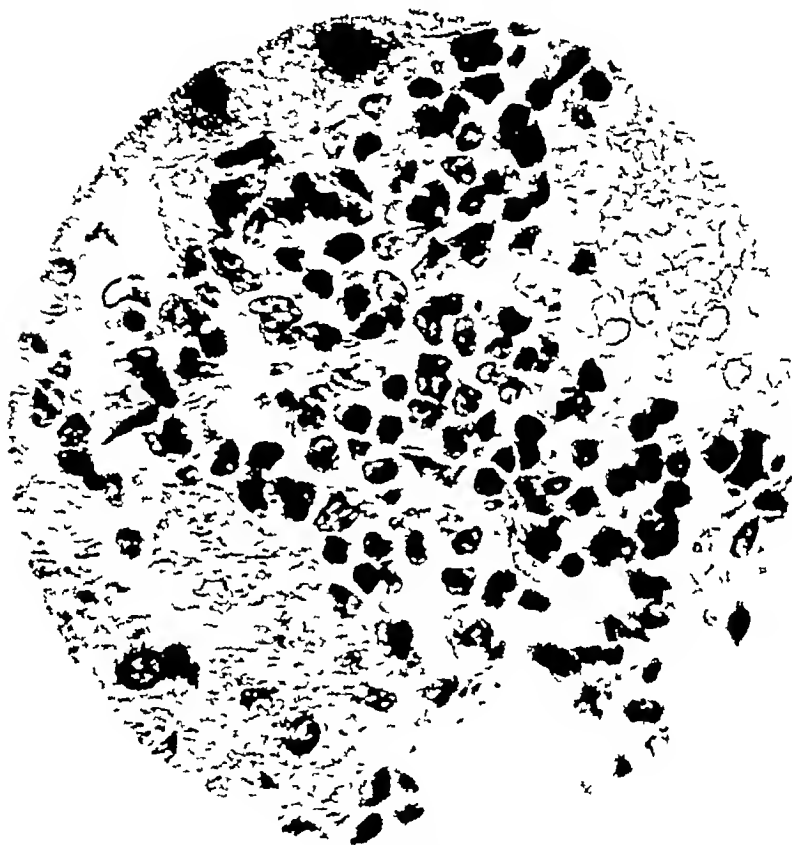


FIG. 2. LYMPHOID REACTION
Infiltration of liver with lymphoid cells following injections

The *lymphoid reaction* occurred in varying degrees in all of the 10 guinea pigs injected with extracts of lymphoid leukemic tissues and consisted of

- 1 hyperplasia of the lymph nodes and follicles of the spleen,
- 2 diffuse infiltrations of lymphoid cells in the liver particularly about the blood vessels or in the periportal spaces (fig 2)
- 3 scattered infiltrations throughout kidneys but less in adrenals,
- 4 and normal bone marrow findings

In table 2 are listed the type and degree of cellular changes observed after extracts of organs of 1 case of Hodgkin's disease had been injected, also of heart

disease, etc. These *Hodgkin's reactions* were very much less definite than those obtained when extracts of urine of patients with Hodgkin's disease were injected, and consisted of

- 1 small areas of monocytes, polymorphonuclears, and lymphocytes scattered throughout the liver and kidneys,

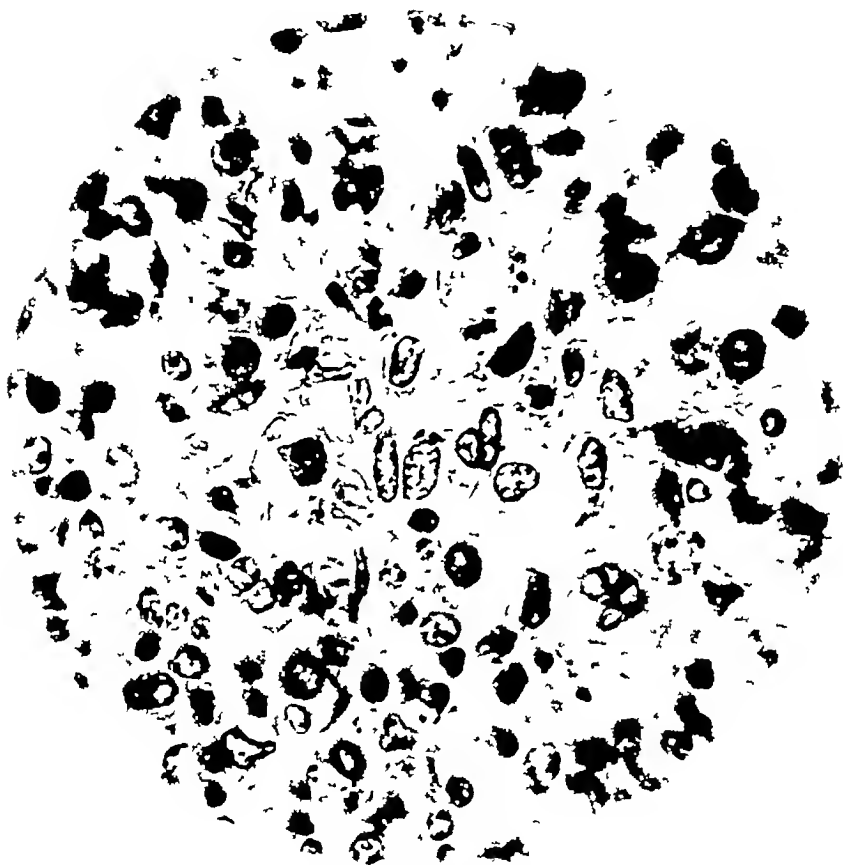


FIG 3 HODGKIN'S REACTION
Cellular changes occurring in lymph nodes following injections

- 2 some of the lymph nodes contained numerous eosinophiles and some hyperplasia of the reticulum cells (fig 3),
- 3 no Sternberg Reed cells were found,
- 4 and normal bone marrow findings

There were no distinctive reactions in the organs of the guinea-pigs injected with extracts of the livers of 3 cases dead of heart disease and 1 of cerebral tumor—the control cases. There were occasional small focal areas of infiltration of lymphocytes or monocytes in various organs, but not of the magnitude of the reactions described above.

There were uncontrollable variations in the interval of time between injection and death of the guinea-pigs and possibly in the nature of the extracts because of variations (autolysis, etc.) in the tissues extracted. We also have assumed that none of the active material responsible for the biological effect was lost in the purification. We have found by previous tedious experience that each guinea pig should have the extract of at least 250 grams of organ and that each animal should be killed in less than 6 weeks after the initial injection. Six guinea-pigs that received extracts made from less than 100 grams of organ from patients with myeloid leukemia did not exhibit the characteristic myeloid reaction. Also there were some animals that died in less than 1 week after the initial injection. Perhaps there was insufficient time, in these cases, to permit cellular reactions to occur.

The fractions of the extracts used were of similar chemical type to those of Turner and Miller. This suggests that the biological reactions observed here are due to the same acids found by Turner and Miller in urine and feces of patients with the various diseases of the leukemic group.

SUMMARY

By the injection of lipid extracts of organs (liver, spleen, and lymph nodes) of 5 patients with leukemia, 1 with Hodgkin's disease, and of 4 control cases (3 of heart disease and 1 of cerebral tumor) into 28 guinea-pigs, it was observed that

- a extracts from human myeloid leukemic organs produced myeloid reactions or infiltrations in guinea-pigs,
- b extracts from lymphoid leukemic organs produced lymphoid reactions¹,
- c extracts from organs involved with Hodgkin's disease produced Hodgkin's reactions², and
- d extracts from normal organs produced slight lymphoid or monocytoid infiltrations.

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MONOCYTIC LEUKEMIA AND TUBERCULOSIS

B₃ ELWYN L. HELLER, M D , AND CHARLES H. HILES, M.D

SINCE the recognition of a monocytic type of leukemia following the report of Reschad and Schilling-Torgau¹ in 1913, enough cases have been observed to warrant its acceptance as an entity distinct from the myeloid and lymphatic varieties²⁻⁵

The present case is of particular interest because it was associated with an active tuberculous infection, the possibility that the hematologic findings may have represented a leukemoid reaction secondary to a widespread tuberculous infection became a major consideration

REPORT OF A CASE

H L , a 70 year old retired clergyman, was admitted to the Eye and Ear Hospital of Pittsburgh on September 28, 1945, complaining of severe sore throat, persistent nasal obstruction, frontal headaches, and a low grade fever of 10 days duration Sinus trouble had been present for several years Past history included an appendectomy and incision and drainage of a tuberculous abscess in the lumbar region in 1939, which drained for many months thereafter A guinea pig inoculated with the exudate developed generalized tuberculous infection

Physical examination revealed no sinus tenderness a swollen, boggy, and crusted nasal mucous membrane, acutely inflamed tonsils, and a granular injected pharynx Except for pallor and slight pyorrhea alveolaris the gums appeared normal The blood pressure was 110/65 The physical findings were otherwise normal

The urine was essentially normal The erythrocytes were 3,030,000 per cu mm , the hemoglobin was 75 per cent, the white blood cell count was 11,950 per cu mm Abnormal cells were noted in the blood smear, which was subsequently examined and diagnosed as monocytic leukemia by Dr Mortimer Cohen *

A dental consultant advised extraction of the teeth, but the procedure was considered inadvisable because of the patient's weakened condition and the hematologic findings Therapy with penicillin, begun on admission and continued for 9 days, was discontinued after a total dose of 1,040,000 units The patient was transferred to the service of Dr R R Snowden at the Presbyterian Hospital on October 16, 1945 He complained chiefly of weakness, and his condition rapidly deteriorated Lethargy, pallor, and mental depression were extreme Pleural effusion, pleural and pericardial friction sounds were noted several days before death, which occurred on November 14 1945, less than 7 weeks from the admission to the hospital

Röntgenologic examination of the chest on October 20 revealed enlargement of the left ventricle, bilateral pleural effusion, and calcified nodes at the lung roots

The laboratory findings were essentially normal, excepting those of the blood The results of several blood counts are recorded in table 1 Thirty-one per cent of the leukocytes were positive to the oxydase reaction Platelets on November 6 were 93,200 per cu mm Smears of sternal marrow obtained by needle aspiration revealed numerous monocytic cells many morphologically typical of mature monocytes, mingled with numerous myeloid and nucleated red cells

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* Died June 20, 1946

POSTMORTEM FINDINGS

The autopsy was performed 2 hours after death. The body appeared wasted, the skin was sallow and numerous petechial hemorrhages were noted over both flanks. The axillary and inguinal lymph nodes were discrete, moderately enlarged and rounded. The edge of the liver was palpable 9 cm. below the lower costal border. An incisional scar in the right lumbar region was well healed. There were no other noteworthy abnormalities on external examination.

The pleural cavities each contained approximately 700 cc. of clear watery fluid. The pleural surfaces were irregularly thickened, scarred, edematous, and studded with pale milium nodules. There were numerous fibrinous and fibrous adhesions between the parietal and visceral pleura.

The lungs were voluminous and poorly aerated. On section moderately extensive congestion and edema were noted, most pronounced in the dependent portions. The pericardium was thickened, fibrous and its cavity was almost totally obliterated by adhesions. The spleen weighed 405 grams and was uniformly bluish purple. On section the parenchyma was uniformly soft and reddish brown. The liver weighed 1890 grams. It presented no gross abnormalities. On section of the right kidney thick purulent exudate escaped from the cavity of the pelvis. A papillary tumor involved approximately half of the

TABLE I

Date, 1945	R.B.C.	Hb	W.B.C.	P.M.N.	Myelo-cytes	Lym-pho-cytes	Mono-cytes	Pro-mono-cytes	Blasts	Additional
	millions	%		%	%	%	%	%	%	
Oct. 19	2.90	60	32,500	21		7	64	8	0	Occasional nucleated R.B.C.
Oct. 23	2.50	56	43,000	21	4	6	60	5	4	Occasional nucleated R.B.C.
Oct. 26			57,600	56		10	29	5	0	
Oct. 29	2.42		59,500	17	3	2	76	2	0	4 nucleated R.B.C.
Nov. 1			44,000	28		8	46	18	0	11 nucleated R.B.C.
Nov. 3			58,500	19	3	0	74	1	3	9 nucleated R.B.C.
Nov. 6	2.15	54	55,900	16	1	4	78	1	0	2 nucleated R.B.C.
Nov. 8			72,000	19	1	5	66	8	1	7 nucleated R.B.C.
Nov. 13			100,500	23	2	6	44	16	9	14 nucleated R.B.C.

inferior pelvic surface, it did not appear to extend into the overlying renal medulla. The ureteral orifice at the ureteropelvic junction was occluded by the tumor.

The axillary, inguinal, peribronchial, mediastinal, mesenteric, and paravertebral lymph nodes were moderately enlarged. Nodes of the latter group measured up to 4 cm. in diameter. They were discrete, their capsules were thin, tense, and intact. On section the pulp was solid, pale, and fleshy. No areas of degeneration or necrosis were apparent.

The marrow of the vertebral bodies and ribs was uniformly deep red and hyperplastic in appearance. The bony trabeculae of the spongiosa were softened and cut readily.

There were no additional gross abnormalities of significance.

MICROSCOPIC EXAMINATION

Lungs. The pleura was thickened by fibrous tissue containing typical nodular tubercles frequently well organized by scar tissue. In areas there was a heavy diffuse infiltration with large cells of monocyte type, many of which appeared immature. The cytoplasm was abundant, pink, nongranular, and frequently irregular in contour, the nuclei were large, vesicular, and frequently lobate. In the pulmonary parenchyma there were numerous perivascular infiltrations with monocyte cells which frequently had caused considerable thickening of the alveolar walls. Immature forms were numerous. Serous fluid distended many of the alveolar spaces, which contained mature monocytes in small numbers. Purulent

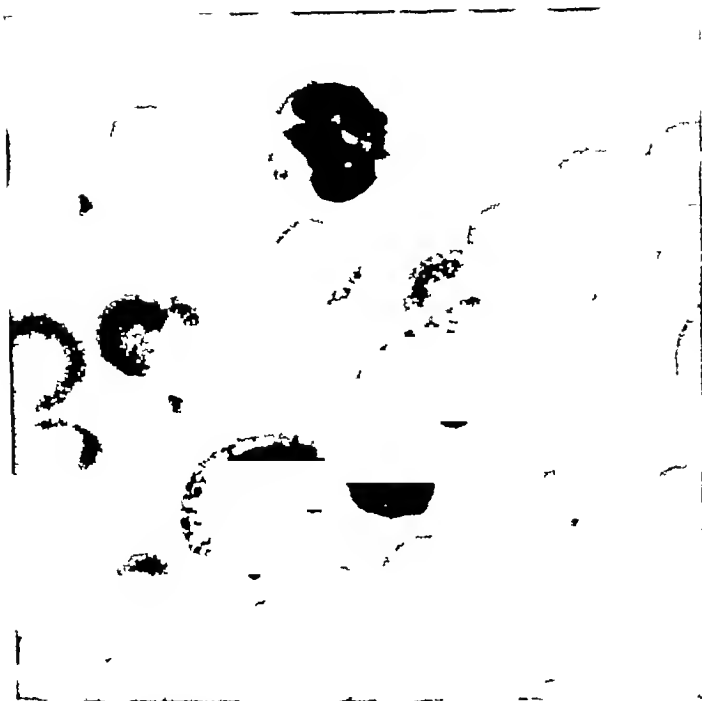


FIG 1 BLOOD SMEAR SHOWING TWO MONOCYTES AND A NEUTROPHIL $\times 1250$

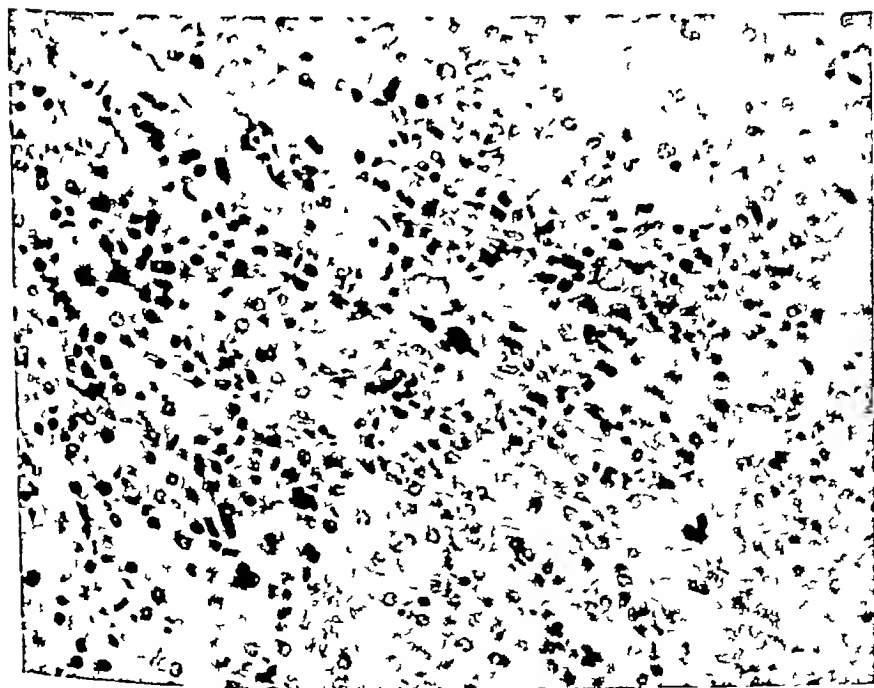


FIG 2 LIVER SHOWING DIFFUSE CELLULAR INFILTRATION AND COMPRESSION OF THE HEPATIC CORDS $\times 300$



FIG 3 LYMPH NODE MILIARY TUBERCLES ARE SURROUNDED BY INFLAMMATORY AND FIBROBLASTIC TISSUE.
X 120

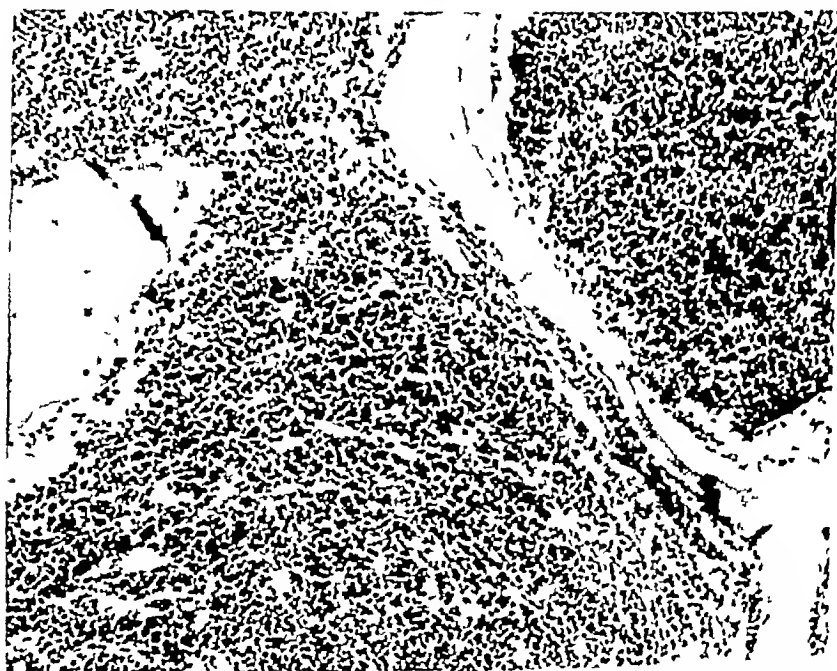


FIG 4 BONE MARROW THE CELLULAR PROLIFERATION HAS REPLACED THE FAT AND HEMATOPOIETIC TISSUE
Note compression atrophy of the bone lamella X 120



FIG 5 TESTIS SHOWING DIFFUSE INTERSTITIAL INTILTRATION $\times 130$

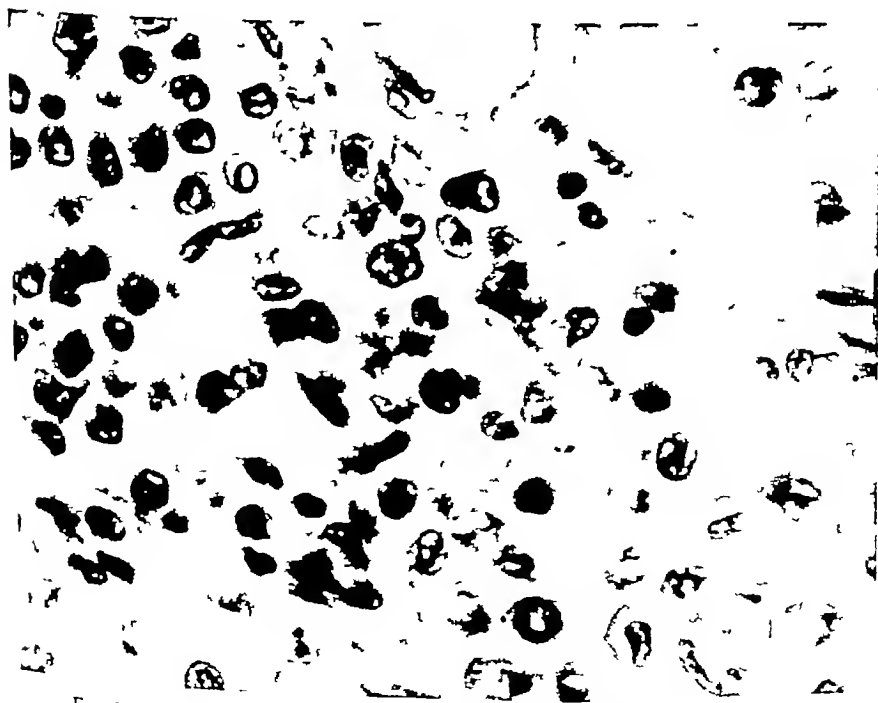


FIG 6 TESTIS THE MONOCYTIC CHARACTERISTICS OF THE CELLS ARE EVIDENT $\times 800$

exudate was present in the lumen of a few bronchioles, surrounding which were small pneumonic foci. No tuberculous involvement of pulmonary parenchyma was demonstrable.

Heart The epicardium was considerably thickened by vascular scar tissue within which were nodular, well-organized tubercles. Throughout the myocardium were numerous small irregular interstitial infiltrations with cells of the monocytic series which were present in considerable numbers in the vascular lumina. The tuberculous reaction had not extended beneath the epicardium.

Spleen The pulp was unusually cellular but the essential structure of the organ was not significantly altered. The lymphoid follicles were distinct and frequently atrophic. Throughout the interstices of the pulp were mononuclear cells of monocyte type, so numerous as to cause compression and distortion of the sinusoidal spaces. They intermingled with smaller numbers of lymphoid cells and scattered plasma cells and neutrophilic leukocytes. Within the sinusoids in small numbers were monocytic cells which were morphologically indistinguishable from the reticulo-endothelium. No tuberculous involvement was apparent.

Liver In some of the portal areas there were small infiltrations with monocytic cells. Identical cells were present in profusion throughout the sinusoids, in some regions causing compression and atrophy of the hepatic cords. The Kupffer cells generally were hypertrophied and could be distinguished from the monocytic cells merely by their position along the sinusoidal walls. There was no suggestion of a tuberculous reaction.

Kidneys Interstitial infiltrations of the parenchyma with mononuclear cells were numerous. They were generally perivascular. The pelvic epithelium of the right kidney had been converted into an extensive neoplasm composed of closely approximated papillary projections covered with epithelium of transitional cell type showing stratification, loss of polarity, and scattered mitotic figures. There was no sign of invasion of the renal parenchyma. There was no evidence of tuberculosis in either kidney.

Lymph Nodes Sections of the nodes from various regions revealed an extensive inflammatory reaction exhibiting typical miliary and small conglomerate tubercles, and small foci of caseous necrosis throughout a fibroblastic matrix.* In some nodes, uninvolved by the tuberculous process, there was a diffuse infiltration of the pulp tissue with cells of the monocytic series, which frequently extended into and through the capsule into the perinodal fat. The reticulo-endothelial cells were hypertrophied, frequently epithelioid, and appeared free in the sinusoidal lumina in moderate numbers.

Bone Marrow Sections of the marrow of several of the vertebrae and a rib showed a diffuse massive cellular proliferation strongly suggestive of a malignant process. It had resulted in almost total replacement of the marrow fat and had caused narrowing and atrophy of the bony trabeculae. The proliferation formed no structural pattern and the cells were relatively uniform in size and appearance. They had round, oval reniform, or lobate nuclei generally vesicular but occasionally in mitotic division. The cytoplasm was prominent, acidophilic, nongranular, and at times irregular in outline. The vascular spaces were for the most part obliterated. Megakaryocytes were seen infrequently. Small nests of erythropoietic and myeloid tissue were occasionally encountered. There was no necrosis or inflammatory reaction.

Miscellaneous Sections of the thyroid, aorta, gastro-intestinal tract, pancreas, bladder, prostate, adrenals, and testis showed heavy intravascular and perivascular collections of monocytic cells many of which appeared immature. None of these tissues showed evidence of a tuberculous reaction.†

DISCUSSION

The combination of the clinical and hematologic features of the reported case stimulated a vigorous controversy among the several attending physicians and consultants which was not completely settled by the results of the postmortem examination. One group interpreted the hematologic findings to be those of a leukemoid reaction complicating a tuberculous infection. This interpretation was

* Two guinea pigs, inoculated with an emulsion of lymph node, contracted tuberculosis.

† Tubercle bacilli could not be demonstrated in sections of liver, spleen, and bone marrow.

based upon several factors: 1 the known tuberculous infection of the patient, proven bacteriologically, several years before the terminal illness, 2 clinical evidence of activity of the tuberculosis indicated by the pleurisy with effusion and the pericarditis, 3 the specific mobilizing effect of the tubercle bacillus on the monocytes, 4 the occurrence of leukemoid reactions in tuberculosis. Indirectly the rarity of monocytic leukemia strengthened the leukemoid position.

The contrary opinion regarded the hematologic findings as specifically diagnostic of monocytic leukemia.

After a review of the clinical, hematologic, and postmortem findings several considerations led us to exclude a leukemoid reaction in spite of the fact that an active and extensive tuberculous infection not only existed but contributed to the death of the patient.

In general, leukemoid reactions represent alterations in the blood picture, usually the result of an infectious process.⁶ Unusually high leukocytosis and the appearance of immature leukocytes in the blood smear may simulate leukemia but, as a rule, repeated competent hematologic study can differentiate such reactions from leukemia.⁷ Pathologically one rarely encounters cases, adequately studied postmortem, in which differentiation offers any serious difficulty. The massive hyperplasia and anaplasia of the hematopoietic tissues in leukemia and the widespread cellular infiltrations of viscera are characteristic and specifically diagnostic. Admittedly rare cases are seen in which absolute differentiation between genuine leukemia and leukemoid reactions is not possible even after thorough postmortem study.^{6, 8} We do not consider our case to belong in this category.

It is well known that elevation in the percentage of monocytes in the blood may occur in tuberculosis⁹⁻¹¹ and that these cells are the most typical and characteristic in the inflammatory response of the host tissues. To our knowledge a monocytic reaction of the blood similar to, or even closely approaching, that of our case has never been described in tuberculosis or any other infectious disease. Leukemoid reactions are rare in tuberculosis¹¹ and they are predominantly of myeloid type,¹²⁻¹⁴ rarely lymphatic.¹⁶ In respect to monocytic reactions in infectious processes, Evans states,⁵ "Descriptions of prolonged monocytosis of significant proportion and more particularly of all stages in the life cycle of the monocyte are not available." The presence of promonocytes and blast forms in our case adds considerable weight to the leukemic interpretation.

The few reported cases of leukemia complicating tuberculosis are almost invariably of myeloid or lymphatic type.¹⁶⁻¹⁸ This is consistent with expectations based upon the relative frequency of the three major types of leukemia and is probably a reflection of an accidental association of the two presumably unrelated diseases. One cannot exclude the possibility, however, that the tuberculous infection may have stimulated an irreversible hyperplasia of the reticulo-endothelial cells, i. e., monocytic leukemia. Kirshbaum and Preuss¹⁹ reported co-existent tuberculosis in 13 per cent of 123 cases of leukemia of all types studied postmortem. We have encountered in the literature only one case of active tuberculosis associated with monocytic leukemia.²⁰

We consider all of the criteria requisite for the diagnosis of monocytic leukemia

to have been fulfilled in our case. These criteria include 1 the progressive elevation of the white blood cell count, exceeding 100,000 per cu mm, with monocytes and immature forms of the monocytic series constantly present in high percentage, 2 the character of the reaction of those lymph nodes which were free of tuberculosis, 3 the massive cellularity and anaplasia of the bone marrow, 4 the widespread intravascular aggregates and the heavy perivascular and interstitial infiltrations of monocytic cells in viscera uninvolved by the tuberculous process.

Clinically the rapid course of the illness was typically that of monocytic leukemia resulting in death about two months after the onset of symptoms. The absence of the gingival lesions frequently ascribed to monocytic leukemia is consistent with the experience of Watkins and Hall.⁴

In our opinion the failure to study the supravital staining reaction of the monocytes of the blood does not render their identity questionable. In the blood smears the majority of the cells were morphologically typical monocytes, and transition stages between mature and blast forms were distinct. Of interest in this respect is the fact that there was complete unanimity of opinion among the numerous hematologists and pathologists who studied the blood smears that the cell in question was a monocyte. The characteristic appearance of the cells in the tissue sections also indicated their monocytic nature.

The possibility that the patient terminally developed acute hematogenous dissemination of the tubercle bacilli, and that the widespread perivascular and interstitial monocytic infiltrations of the viscera were early cellular reactions to the bacteremia, has been considered. However, the general character of the cellular infiltrations, and the absence of both focal necroses and the various developmental stages of miliary tubercles in the viscera, so typical of tuberculous bacteremia, negate such a possibility.

SUMMARY

A case of monocytic leukemia associated with active tuberculosis of pleura, pericardium, and lymph nodes is reported.

The criteria for the diagnosis of monocytic leukemia and the factors excluding a leukemoid reaction are presented.

The rarity of monocytic leukemia in contrast with the frequency of tuberculous infections and the rarity of active tuberculosis in the reported cases of monocytic leukemia indicate that the association of the two diseases is probably coincidental.

Grateful acknowledgment is due to Miss Anne Shiras and Mortimer Cohen, M.D., for the photomicrographs.

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PLASMA CELL LEUKEMIA

By WILLIAM T. MOSS, M.D., AND LAUREN V. ACKERMAN, M.D.

THE following case appears to be a typical example of plasma cell leukemia, which because of its rarity deserves reporting.

REPORT OF CASE

Case Summary A G, a white man, aged 63, entered the hospital October 27, 1944, complaining of increasing fatigue, anorexia, and weight loss of 3 months duration. Two months prior to entry a swelling of the right lower jaw developed. This was incised by a local dentist, and the resulting hemorrhage was severe. Following this, numerous hemorrhages from the nose and gums occurred, and tarry as well as blood streaked stools were noticed. Slight exertion produced sharp pains in the right lower anterior chest wall.

Physical Examination (Oct. 31, 1944) The patient was a well-developed but acutely ill male who was weak, dyspneic, and pale. There were petechiae over the posterior aspect of the legs. There was no superficial lymph node enlargement. The gums were swollen and bleeding, and clotted blood was present in both nostrils. There was no tenderness on pressure over the sternum or ribs, despite a history of recent rib fractures. In the right axilla there was a draining, recently incised furuncle. The abdominal examination showed fullness in both upper quadrants, more marked on the left. The splenic and liver margins could be felt to descend 6 cm. below the costal margins. There was no clinical evidence of ascites.

Laboratory Data Urinalysis was negative, no Bence Jones protein was present at admission or 3 days prior to death. The Kahn reaction was negative. Nonprotein nitrogen was 20 mg. per cent, total serum proteins, 7.4 Gm., with albumin 2.6 Gm., globulin 4.8 Gm., and an albumin/globulin ratio of 0.5. Blood count (Oct. 31, 1944) red blood cells 2,120,000 per cu. mm., hemoglobin, 7 Gm., white blood cells, 13,700, platelets, 330,000, differential: myelocytes, 4 per cent, juveniles, 14 per cent, stab, 15 per cent, segmented polymorphonuclear neutrophils, 24 per cent, lymphocytes, 23 per cent, monocytes, 6 per cent, eosinophils, 2 per cent, basophils 1 per cent, and plasmocytes, 11 per cent with 4 per cent pronormoblasts and 24 per cent normoblasts. This count varied only slightly throughout the entire course of the disease, in spite of numerous transfusions. The sternal bone marrow (Nov. 1, 1944) showed the marrow completely replaced by plasma cells.

A skeletal x-ray showed a moth-eaten appearance in several ribs, more marked in the anterior ends of the third, fourth, fifth, and sixth ribs on the right, and in the acromial ends of both clavicles. The skull was not remarkable. X-ray of the dorsal and lumbar vertebrae was unusual only because no trabeculae could be visualized.

Course On the basis of the clinical and hematological findings, a diagnosis of plasma cell leukemia was made. The plasma cells in the peripheral blood increased to 17 per cent of the leukocytes 6 days after the patient was first seen, and to a maximum value of 22 per cent 2 weeks later (fig. 1). The hyperglobulinemia remained essentially unchanged throughout the disease. The patient received 11 blood transfusions of 500 cc. each during his hospital stay, and teleroentgentherapy was given for 13 days (10 doses of 10 r. each), with slight temporary improvement. He expired 1½ months after admission to the hospital, and 5 months following onset of symptoms.

NECROPSY REPORT

The body was that of an edematous, anemic looking, white male. The skin had numerous 0.2 to 0.3 cm. petechiae scattered over the entire body. In the peritoneal cavity there was 6 liters of thin bloody fluid and on the peritoneal surface were numerous additional hemorrhages. There was 750 cc. of clear yellow fluid in the left pleural cavity, but the right was free of fluid. The heart weighed 280 grams. The lungs were moderately congested. The spleen weighed 665 grams and its architecture was poorly defined. The pancreas weighed 120 grams and contained many grayish white areas 0.5 cm. to 1 cm. in diameter.

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where the architecture was obliterated. The liver weighed 2500 grams and on cut section its normal architecture was present. There were numerous round, yellowish, poorly defined areas measuring 1 mm to 2 mm on its cut surface. Enlarged axillary, peribronchial, periaortic, celiac, and mesenteric nodes



FIG. 1 BLOOD SMEAR

Note characteristic appearance of plasma cells. Insert reveals typical form with eccentric nucleus, perinuclear halo, and usual arrangement of the chromatin.

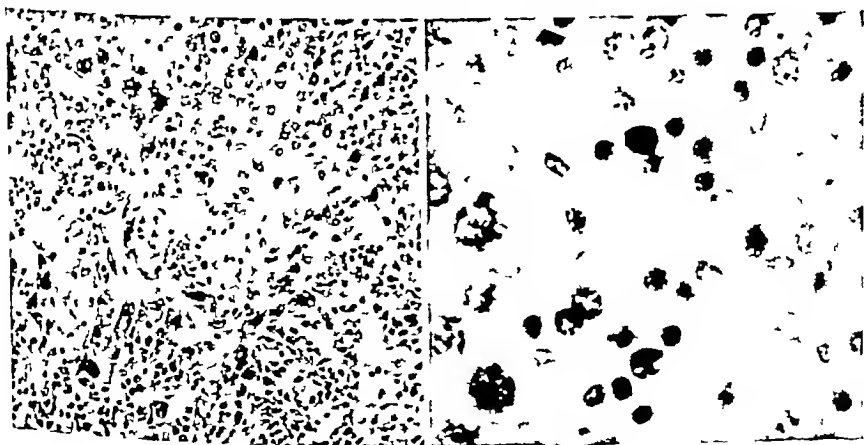


FIG. 2

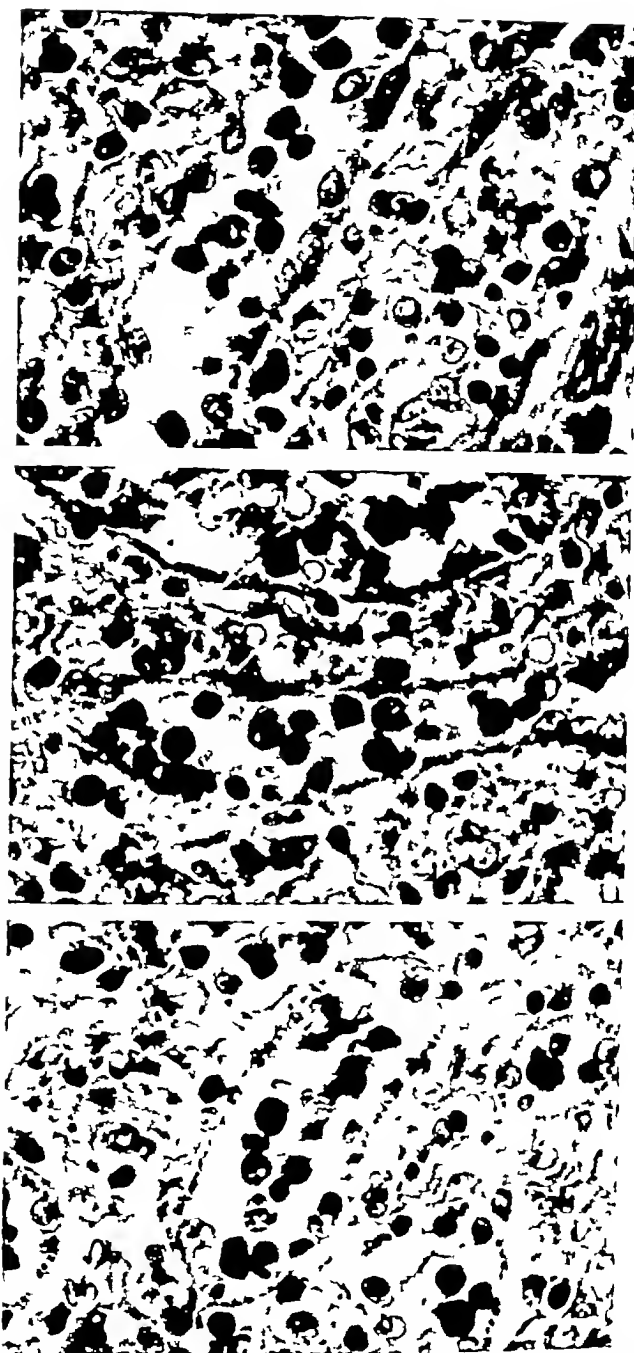
FIG. 3

FIG. 2 (LEFT) DIFFUSE INFILTRATION OF LIVER RESEMBLING THAT OBSERVED IN MYELOGENOUS LEUKEMIA $\times 135$

FIG. 3 (RIGHT) LIVER SHOWS NUMEROUS PLASMA CELLS WITH ONE BINUCLEATED FORM. RETICULO-ENDOTHELIAL ORIGIN IS SUGGESTED $\times 1400$

were seen. They had a soft consistency and the largest of these measured 1.5 cm in diameter. In the bodies of the dorsal and lumbar vertebrae were several 2 mm, whitish areas.

Microscopic Examination. Liver Plasma cells were present in every region of the liver, but were more



FIGS 4 (TOP), 5 (CENTER) AND 6 (BOTTOM) SPLEEN DEMONSTRATES NUMEROUS BLOOD VESSELS WITH PLASMA CELLS IN VARIOUS STAGES OF DEVELOPMENT. RETICULO-ENDOTHELIAL ORIGIN IS SUGGESTED
X 1400

marked around the liver lobules and the central veins. In the sinusoids and between individual cells there were also numerous plasma cells (figs. 2 and 3). These cells gave no sign that they arose from Kupfer cells and in no way resembled them. Areas of duct proliferation were seen within all focal collections of plasma cells. More multinucleated forms were found in the liver than in any other organ, occasionally as many as 4 nuclei were found in a single cell. Many large and small blood vessels in the liver, spleen, and pancreas showed large clumps of peripherally placed plasma cells in their lumina.

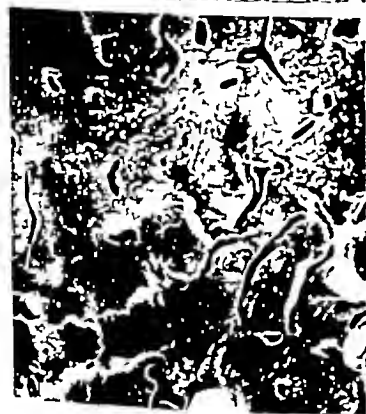
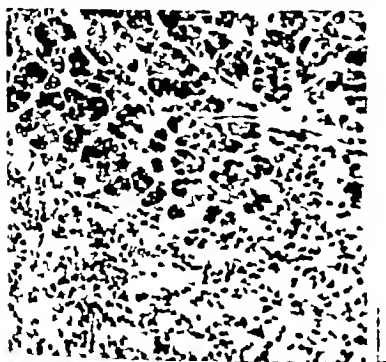


FIG 7 (UPPER LEFT) DIFFUSE INFILTRATION OF PANCREAS $\times 135$

FIG 8 (LOWER LEFT) BONE MARROW REVEALS DIMINUTION AND REDUCTION IN SIZE OF BONE SPICULES (LOW POWER)

FIG 9 (UPPER RIGHT) BONE MARROW

Note almost complete replacement by plasma cells in various stages of formation and their close proximity to bone $\times 1400$

FIG 10 (LOWER RIGHT) ROENTGENOGRAM OF TWO VERTEBRAE EXCISED AT AUTOPSY

Note coarse lacy pattern suggesting osteoporosis but due to diffuse replacement by plasma cells

Spleen The normal architecture of the spleen was almost completely destroyed. The sinuses were plugged with plasma cells while the white and red pulp had largely been replaced by them. There were, however, some collections of lymphocytes in the red pulp which were well demarcated and easily distinguished from the adjacent plasma cells. Nothing resembling transitional cells between these two cell types could be identified. It could not be ascertained that plasma cells adjacent to the sinus endothelium were arising from this endothelium, but the relation of the plasma cells to the endothelium was certainly suggestive (figs. 4, 5, and 6). Occasionally plasma cells could be seen partially migrating through small clefts in the sinus wall.

Lymph Nodes Two celiac and one peribronchial node showed complete destruction of their structure by plasma cells which invaded the capsule and surrounding fat. The sinuses were plugged with the cells and there was no sign of persisting follicles. Evidence that the plasma cells arose from the few scarce lymphocytes could not be found.

Pancreas Plasma cells had infiltrated between the lobules and acini and even into the surrounding fat (fig. 7). An estimated one fourth of the acini had been destroyed. The islets were not invaded although they were often well surrounded by plasma cells.

Bone Marrow The bone marrow from the sternum, ribs and vertebral bodies was grossly not remarkable. Microscopically there was an almost complete replacement of normal elements by a diffuse infiltration of plasma cells. The trabeculae of the spongy bone were decreased in size and number (fig. 8) and plasma cells were in direct contact with trabeculae as described by Geschickter (fig. 9). An x-ray was taken of a segment of lumbar vertebral bodies removed at postmortem examination. This revealed a coarse lacy pattern with markedly diminished trabeculae (fig. 10).

There were focal collections of plasma cells in the perirenal and periadrenal fat, the seminal vesicles and the testicles. There were no important changes in the heart, thyroid, prostate, gallbladder, kidneys, stomach, esophagus, aorta, small or large bowel, lungs or lumbar axillary or peribronchial lymph nodes.

Microscopic Diagnosis Plasma cell leukemia with diffuse bone marrow involvement and with infiltration of the liver, spleen, pancreas, lymph nodes, testicles and seminal vesicles. The diagnosis of plasma cell leukemia was confirmed by five eminent pathologists and hematologists.³

REVIEW OF THE LITERATURE

In the literature there have been 36 cases of plasma cell leukemia⁴⁻³⁴. The first case was reported by Foa (1902), who called the disease pseudoleukemia plasmocellularis.⁴ He found plasma cells in the blood and diffuse infiltration of the bone marrow, liver, spleen, and in some lymph nodes. In our discussion, certain questionable cases have been omitted. Foord's cases 1 and 2³⁵ in many respects resemble plasma cell leukemia, and perhaps by some would be included in this review. Geschickter² has pointed out that in multiple myeloma it is not surprising to find occasional plasma cells in the blood, in view of the fact that myeloma cells can be found in the vessels in the tumor area. The consistently low counts present in Foord's cases, the clinical signs and symptoms of multiple myeloma rather than of leukemia, and the fact that Foord himself has failed to classify his cases as plasma cell leukemia, have led us to omit them from this review. The case reported by Lucksch³⁶ has also been omitted because the data were incomplete and the bone marrow biopsy was negative.

CLINICAL FINDINGS

Age Plasma cell leukemia has been reported in patients from 15⁹ to 78¹ years of age, but is usually found between the ages of 45 and 60. Sixty per cent of the cases occur after 50. The average age of all cases reported, excluding the single 15 year old child, is 54.2 years.

Sex In contrast to multiple myeloma in which 70 per cent are males,² neither sex shows a predisposition for plasma cell leukemia. Of the 32 cases reporting sex, 16 were males and 16 were females.

Clinical Manifestations The early symptoms of plasma cell leukemia in the majority of cases are those of multiple myeloma. The most frequent initial symptom is vague pain generally beginning in the back. However, it may be in the arm,¹ legs,¹⁴ or substernal region.¹³ Accompanying or preceding the pain there is usually

a history of lassitude, weakness, and weight loss. Anemia also frequently develops, which intensifies the last-mentioned symptom and may produce tingling sensations in the extremities.²¹ Vertebral lesions may produce neurological signs and symptoms.²¹ In cases where no primary osseous lesions can be located, tendency to hemorrhage may bring the patient to the hospital.¹⁴ Thirteen cases in addition to our own have shown hemorrhagic tendencies. The bleeding is nearly always from the gingiva or nose, but hemorrhage from the gastric mucosa was reported by Askanazy.²³ These hemorrhages frequently occur near the termination of the disease.

The bone-destructive nature of plasma cell leukemia is exemplified by frequent pathologic fractures. Five of the 36 cases had such fractures.

Pathology. The usual pathologic picture produced by plasma cell leukemia is not grossly different from that of any chronic or subchronic leukemia, with the addition of multiple myelomatosis. Meyer²⁴ found in the literature 22 cases (including one by Lucksch¹⁶) of multiple myeloma with plasma cells in the circulating blood. To this list may be added our own, one case by Lindeboom,²¹ and one by Keilhack.³⁰ Maresch⁵ and Hertz¹¹ have described extraskkeletal plasmocytomas as primary lesions in their cases. When Osgood and Hunter¹⁵ reported a case in 1934, they found only their own and Piney's case¹⁴ without evidence of associated tumor. Meyer²⁴ believes his case also falls in this group. However, as Patek²¹ has pointed out, the meticulous roentgen ray study and search at autopsy for plasmocytoma necessary to exclude the presence of tumors was not made in the first two of these cases. Consequently, associated plasmocytoma in plasma cell leukemia should be suspected until every possible method of exclusion has shown it to be absent.

As in other leukemias, there is systemic involvement of the hemopoietic system. Thus, the bone marrow, spleen, liver, and lymph nodes are usually much altered.

Bone Marrow. In plasma cell leukemia the bone marrow shows diffuse infiltration. This finding is essential for a diagnosis of plasma cell leukemia. The plasma cells found in the bone marrow do not differ from those in the blood, except that in the blood the plasma cells tend to be smaller.³⁰ The spongy bone is destroyed just as it is in multiple myeloma, but as Keilhack had pointed out, osteoclasts apparently have no part in this process. Geschickter² described this same destructive process in the punched-out areas of uncomplicated multiple myeloma. Our photomicrographs of diffusely infiltrated bone marrow show a decrease in both the number and the size of the trabeculae. The roentgen ray manifestation of spongy bone destruction shows a coarse lacy pattern somewhat resembling osteoporosis. However, even where the marrow is infiltrated, an apparently normal roentgenogram may be obtained. Also, on gross examination the bone marrow may appear normal when the microscopic examination reveals diffuse infiltration by plasma cells. This is accounted for by the diffuse nature of the infiltration and the persistence of some trabeculae to show no gross alteration in the bone marrow.

Liver. The liver apparently is enlarged in about one-half of the autopsied cases and may weigh as much as 2550 grams,¹⁸ although the average weight is 1900 grams. In the great majority of cases it is invaded by plasma cells. Of 29 autopsied cases in which this factor was mentioned, 25 showed liver involvement. This

involvement is usually one of diffuse infiltration (as in myelogenous leukemia), i.e., in sinusoids, between liver cells, and in the periportal areas.³⁰ However, focal areas of involvement have been noted by Jones and Bruns,²² and by Apitz.²⁹ In our case there were definitely more multinucleated forms in the liver than in the other involved organs. Reiter²⁴ has reported more young forms in the liver than in any other organ.

Spleen The spleen is also usually enlarged and in one case was reported as weighing 1665 grams.²⁹ In 22 of the 26 autopsied cases in which this weight factor was noted, the spleen was above normal weight. Its usual weight, when associated with plasma cell leukemia, is 325 grams to 400 grams. On cut section the spleen usually has a poorly defined or a completely destroyed architecture. The parenchyma, as described and illustrated by Osgood,¹⁸ may show circumscribed but often confluent whitish areas. On the other hand, an enlarged, diffusely infiltrated spleen may present an entirely unremarkable cut section. Microscopically, as a rule, the follicles are completely absent, only occasional lymphocytes are seen, and the sinuses and reticulum are filled with plasma cells. Osgood¹⁸ and Keilhack³⁰ mention the large amounts of hemosiderin in the reticulum cells of the spleen, which are manifestations of blood extravasation.

Lymph Nodes Too frequently a description of adequate lymph node examination in plasma cell leukemia is missing from the case reports. An account of generalized lymphadenopathy has not been found in the literature, but nearly every lymph node area has been invaded by this disease: cervical,^{13 14 23} inguinal,²² axillary,¹⁸ paraportal,¹⁸ and retroperitoneal nodes.²³ Such nodes usually show an absence of germinal centers and a filling of the sinuses with plasma cells.

Pancreas This organ has only occasionally been reported as being infiltrated by plasma cell leukemia. Patek and Castle²¹ found in the tail of the pancreas a tiny nodule composed of plasma cells, and immediately surrounding which the normal pancreatic tissue was invaded. In our case there was diffuse pancreatic infiltration. The islets of Langerhans were spared infiltration and destruction, but the acinar tissue showed both.

Other Organs Numerous other organs have infrequently been infiltrated by plasma cells. The kidney has been reported involved seven times,^{7 14 21 23 24 29 30} usually described as a diffuse infiltration in the interstitial tissue between the tubules. The interstitial tissue of the lungs was reported infiltrated by Keilhack,³⁰ the heart, by Osgood,¹⁸ the tonsil, by Ghon and Roman,¹⁰ the gastric mucosa, by Osgood,¹⁸ the testis, by Ulrich,²⁷ and the skin, prostate, and parotid, by Piney and Riach.¹⁷ Kreibich¹² believed that a skin nodule was the primary lesion in his case, and that the bone marrow infiltration was metastatic. However, Ulrich²⁷ has thought this to be very unlikely.

DIFFERENTIAL DIAGNOSIS

Askanazy³³ has formulated the following as requirements for a diagnosis of plasma cell leukemia:

- 1 Plasma cells must be present in the peripheral blood (more than just terminally)

2. Leukocytosis

3. Diffusely involved bone marrow

This list makes no provision for the aleukemic phase which, as Keilhack³⁰ has pointed out, depends largely on the bone marrow findings for diagnosis. Such an aleukemic phase probably does not differ from the not uncommon, diffuse myelomatosis in which no circulating plasma cells are present. A bone marrow biopsy showing diffuse plasma cell infiltration, together with finding the typical cell in the circulating blood, is necessary and usually sufficient. The clinical signs and symptoms of acute, subacute, or even of chronic leukemia are helpful, but not necessary.

The plasma cells have been reported by Naegeli¹⁷ to increase to 30 per cent in the circulating blood of patients with the measles, in rubella, rubeola, and scarlatina, by Craik,²⁸ in chronic lymphatic leukemia, by Pappenheim,²⁹ and occasionally in myeloid leukemia, metastatic carcinoma, and Hodgkin's disease.²¹ On the other hand, bone marrow infiltration by plasma cells unaccompanied by more than an occasional plasma cell in the circulating blood may be found in hepatic cirrhosis (16 per cent plasma cells), agranulocytosis (21.5 per cent plasma cells), miliary tuberculosis (45 per cent plasma cells), and, to a much less degree, in several hypoplastic anemias.⁴⁰

Laboratory Findings. In plasma cell leukemia the leukocyte count is usually 10,000 to 40,000/mm³. A count of 2900 was reported by Hertz¹¹ and one of 233,400 by Steinhaus,⁹ but these are extremes. A count above 100,000 is very rare. On repeated examinations, the leukocyte count is usually seen to rise slowly as the disease progresses. In 5 of the 36 reported cases, no abnormal elevation of the total white count was observed. The percentage of plasma cells found in the peripheral blood varied in the literature from 87 per cent⁹ to 1 per cent.²⁹ When first seen, an average of 29 per cent plasma cells were found in 29 of the 36 cases presenting these data. The percentage of plasma cells tends to increase slowly as the disease progresses, and conversely, the percentage of neutrophils is found to decrease moderately. In the case reported by Lemaire,²⁸ plasma cells were found in pleural fluid.

The erythrocyte counts at the time the patients were first seen averaged 2,600,000/mm³ (26 of 36 cases), but this count becomes progressively lower. Anemia follows the same pattern as in other leukemias. Forkner⁴² has discussed the etiology of anemia in leukemias and mentions the significance of disorderly blood formation, blood loss, and increased blood destruction. All of these are found also in plasma cell leukemia and undoubtedly account for the anemia present, even though the actual mechanism is not understood. The case reported by Reiter²⁴ was initially thought to be pernicious anemia when the presenting symptoms were marked anemia, weakness, tingling sensation in the fingers, and awkwardness of gait.

The low platelet count so frequently found in plasma cell leukemia is reflected in the high percentage of hemorrhagic tendencies found. As mentioned previously, 13 out of 36 cases have shown abnormal bleeding tendencies. The platelet count was mentioned in 13 cases, described as decreased in 6, and averaged 62,000/mm³ in the other 7. In our case the platelet count was initially normal and remained so during the course of the disease. Thrombocytopenia may account for some of

the hemorrhagic tendencies, but Foord³⁵ has shown similar bleeding in multiple myeloma with normal or slightly reduced platelets, abundant fibrinogen, and an abundance of calcium. He is unable to explain this phenomenon.

Just as in multiple myeloma, hyperglobulinemia is sometimes present in plasma cell leukemia.^{18 21 26 27 34} In addition, a hyperproteinemia was found in Cabot's case.²⁰ The globulin was reported within normal limits in the 2 cases cited by Rubin³² and the 1 by Lemaire.²⁸ Lowenhaupt¹ believes plasma cells are closely related to those cells which are concerned with the formation of antibody globulin. She also believes that in its abnormal form, plasma cells are producers of the hyperglobulinemia.

Bence-Jones Bodies. Of the 37 cases of plasma cell leukemia—which includes our own—the test for Bence-Jones bodies in the urine was performed in 20. Of the 20 tests, 6 were positive, and the rest, negative. Since Geschickter² reported only 65 per cent positive Bence-Jones tests in multiple myeloma, a negative test should obviously not be used to rule out multiple myeloma. Yet both Piney¹⁴ and Os good¹⁸ used it in this fashion. On the other hand, a positive test is good evidence of multiple myeloma, for it is infrequent that Bence-Jones bodies are found in diseases other than multiple myeloma.²

THE RELATIONSHIP BETWEEN MULTIPLE MYELOMA AND PLASMA CELL LEUKEMIA

A series of transitions from typical multiple myeloma to typical plasma cell leukemia has been arranged by Piney and Riach¹ and has been discussed by numerous workers.^{15 18 21 30} The entire series of cases reviewed might be classified more or less in order from those typical of multiple myeloma to those which resemble typical leukemia. However, such a list adds nothing to our knowledge of the disease.

Patek²¹ implied that the more nearly plasma cell leukemia resembled a typical leukemia, the more malignant the disease. Thus the readiness with which plasma cells tend to enter the blood stream should be a measure of malignancy. Such a statement seems logical, but it has not been proven. In multiple myeloma the average duration of life after recognition of symptoms is 2 years. In plasma cell leukemia the average duration of life is 8 months. The cases of plasma cell leukemia with an initial plasma cell count in the peripheral blood of over 50 per cent (4 cases) had an average duration of life of 5 months, while those with an initial plasma cell count under 20 per cent (15 cases) had 8 months' duration of life. With such a small number of cases, not much significance can be attached to these figures, but it does suggest that the readiness with which plasma cells enter the peripheral circulation is a measure of the malignancy.

Since multiple myeloma is a leukocytic tumor, the occurrence of leukemia in certain cases brings up again the close relationship between the leukocytic neoplasms and leukemia, the latter probably being a generalized variant of the former condition.

ORIGIN OF THE PLASMA CELL

Although there has been a great deal of investigation and speculation on the origin of the plasma cell, the question remains unsettled. An extensive review of

the subject is not justified in this paper. Michels¹¹ has discussed the various theories of origin, morphogenesis, function, and development of the plasma cell, and he summarizes the theories of origin as follows: (1) A histogenous origin from connective tissue cells, including tissue lymphocytes, fibroblasts, clasmatoocytes, resting wandering cells, adventitial cells, and hemohistioblasts. (2) A hematogenic origin from emigrated lymphocytes. (3) Mixed origin from emigrated lymphocytes (monocytes) or pre-existent tissue lymphocytes. (4) An origin from immature blood cells (myeloblasts, hemoblasts [erythroblasts, granuloblasts]) through aberration or abortion. Michels favors the third theory. The recent observations of Lowenhaupt¹ are in favor of a reticulo-endothelial origin of plasma cells. She found plasma cells arising from sinus endothelial cells of 7 spleens and concludes:

An origin from histocytic type cells is indicated by the sites of proliferation in the spleen, liver, and lymph nodes, by the type of hepatic infiltration, and is suggested by the invasion of bone. In our case, sections taken from the spleen, liver, and lymph nodes tend to support Lowenhaupt's findings.

SUMMARY

A case of plasma cell leukemia is reported, together with a review of the literature, including a summary of the signs, symptoms, and laboratory findings usually present. The relationship of this leukemic state to ordinary plasmacytoma, a tumor of white cell origin, is discussed.

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MORPHOLOGICAL EXAMINATION OF THE BLOOD AND STERNAL MARROW OF NINE PATIENTS TREATED WITH THIOURACIL

Bj CARL J GESSLER, M D

BECAUSE of the current interest in agranulocytosis following thiouracil administration it was determined to study the blood and bone marrow in a series of 9 patients under treatment with this drug for hyperthyroidism

It is of interest that before treatment was instituted the total leukocyte count in 4 of the 9 cases was less than 4,000 per cu mm, with a correspondingly low value of the absolute number of neutrophils (table 1) Two cases had hemoglobin values under 70 per cent, with a color index of less than 1

EFFECTS OF THIOURACIL ON THE BLOOD

In 2 of the 9 cases treated with thiouracil the leukocyte count dropped below 3,000 per cu mm This does not mean that in our experience leukopenia developed in about 20 per cent of the cases, because actually a larger group of patients was treated with the drug and the only 2 cases which developed leukopenia are included in the smaller group of 9 patients whose bone marrow was examined In a third case (no 2) administration of thiouracil was discontinued because of the development of a severe urticaria, the absolute number of neutrophils fell from 2585 per cu mm at the time treatment was stopped to 1584 18 days later

In 2 other cases (nos 1, 9) toxic neutrophils were seen in the last blood smear, after fairly large amounts of the drug had been given In case no 1, 2 of 61 neutrophils showed a bluish cytoplasm, vacuoles, and coarse granules, in case 9 the only toxic change was a slightly coarse granulation of many neutrophils

In case 2 a blood examination made 3 days before the onset of severe urticaria showed an increase of the eosinophils to 8 per cent Such an increase might be considered as an indication that some form of allergic reaction was becoming manifest

The slight hypochromic anemia in 2 of the cases mentioned above improved in both cases during the administration of thiouracil without any additional anti-anemic therapy

EXAMINATION OF THE STERNAL MARROW AFTER ADMINISTRATION OF THIOURACIL

Puncture was readily performed in all cases except no 6, a 39 year old acromegalic woman with hyperthyroidism (BMR + 48), which was probably of pituitary origin The differential cell count was made according to the technic of Rohr * By this method one does not simply count 500 nucleated cells but enough to obtain 500 cells of the granulocytic and lymphocytic series, the result is expressed in a percentage which permits a more exact comparison between the myelogram and the hemogram

From the hospitals of Brussels Belgium

* Karl Rohr Das Menschliche Knochenmark Leipzig 1940

TABLE 1

Case no	Before Thiouracil			Total amount of thiouracil given (grams)	After Thiouracil		
	Leuko-cytes	Neutrophils			Leuko-cytes	Neutrophils	
		%	Absol number			%	Absol number
1	7,600	32	2,432	32.6 in 66 days	7,100	61	4,331
2	3,100	61	1,891	16.4 in 17 days	4,700	55	2,585 (on 30—\)
					3,600	44	1,584 (on 17—\)
3	8,500	62	5,270	34.3 in 56 days	8,100	79	6,399
4	3,900	60	2,340	36.4 in 68 days	4,800	53	2,544
5	3,900	35	1,365	47.4 in 76 days	2,500	35	875
6	6,700	63	4,221	42.6 in 56 days	3,800	66	2,508
7	3,500	40	1,400	31 in 31 days	2,500	49	1,225
8	4,800	59	2,832	26.1 in 50 days	5,600	63	3,528
9	7,100	57	4,047	61 in 99 days	8,300	64	5,312

TABLE 2—Myelograms

Case no		1	2	3	4	5	6	7	8	9
Reticulum cells	macrophages	0	0	0	0	0	0	0.6	0	0
	plasmacytic	1.6	3	3	1.2	2.8	0.6	2.6	0	2
	lymphoid	0.4	5.2	3.4	0.8	0	0	1.8	2	0.9
Erythro-poiesis	Proerythroblasts	0.2	0.6	0.4	0.2	0.4	0.4	1.2	0	0.4
	Macroblasts									
	basophilic	2.6	2.2	1.6	1	0.8	0.6	1.6	1	4
	polychromatophilic	3.8	2.4	7.8	0.4	4.4	1.6	2.2	7	1
	Normoblasts									
	basophilic	1.2	1	1.4	2.4	1	0.8	1.4	0.7	0
Leuko- and lympho-poiesis	polychromatophilic	20.8	33.4	21.8	16.6	22.6	18.8	35	21.3	4
	orthochromatic	0	1.4	1	0.4	0	0.4	1.2	3.3	1
	Myeloblasts	0	2	0	2.2	0.6	0.8	3	1.7	0.9
	Premyelocytes	6	5.8	3.2	5	9	3.2	11.8	4.4	4.8
	Myelocytes									
	immature	11.8	10.8	9.4	12.4	16	10.6	13	7	11.8
	mature	16	6.8	14.8	10.8	5.4	14.2	6	6	7.6
	Metamyelocytes	9.4	6.4	12	6.6	7.8	18.8	4.4	7.7	7.2
	Neutrophils									
	nonfilament	27	40	7.6	37	41.6	39	36	38.3	35
	filamented	16.8	9	2.6	10.4	8.8	9.8	8.4	18.3	18.4
	Eosinophil myelocytes	3	5.2	6	0	3.6	0	3	4.6	3.4
	Eosinophils	1.4	3	2	0	4	0	3.4	3	2.4
	Basophils	0.4	0.4	0.8	0	0	0.4	0	0	0
	Monocytes	0	0.4	0.8	1	0.2	0	0	0.6	1
	Lymphocytes	7.6	9.8	2	14.4	4.6	4	10.6	8.4	7.6
Granulocyte-erythroblast ratio		3	—	—	9	4	—	—	7	3

The results of our study are given in table 2. In discussing these results, we shall consider the sum of the premyelocytes and immature myelocytes as a unit. In this group are included those cell forms between the myeloblast and mature myelocyte and on whose definition everyone is pretty well agreed. Most striking is the fact that the percentage of premyelocytes + immature myelocytes was found to be markedly increased in the 2 cases (nos. 5, 7) which had developed a leukopenia, as illustrated in the figure. These changes (decrease of the leukocytes and neutrophils in the blood with an increase of the premyelocytes and immature myelocytes in the marrow) are similar to, although less marked than, those seen in many cases of agranulocytosis (disappearance of the neutrophils from the blood and complete predominance of the premyelocytes in the marrow).

Besides the numerical changes there were also some morphological alterations in these 2 cases. In case 7 some of the premyelocytes were abnormally large, many of them had an irregularly shaped nucleus, the same irregularity was noted in a few myeloblasts.

In case 5 some of the premyelocytes had an irregularly shaped nucleus. In both cases one very large premyelocyte was seen with typical blue cytoplasm but a

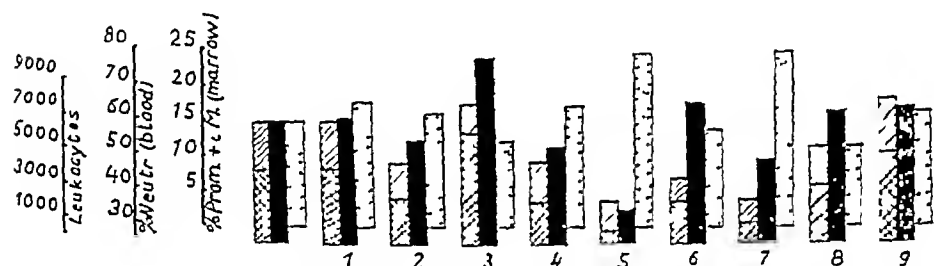


FIG. 1. BLOOD AND BONE MARROW FINDINGS—NORMAL AND THIDURACIL TREATED CASES

Each group of three columns represents the findings in 1 patient (nos. 1-9) except the first one which gives the average normal values. First column: total leukocyte count (hatched) and absolute number of neutrophils (criss-crossed). Second column (solid black): percentage of neutrophils in the blood. Third column (dotted): percentage of premyelocytes + immature myelocytes in the sternal marrow (here we have purposely given, in the first group, the maximum normal value so that all values in excess must be considered abnormal). Note the striking contrast in cases 5 and 7 between the low values of the total leukocyte count and of the number of neutrophils and the increased number of premyelocytes and immature myelocytes.

large nucleus which had the shape of a curved rod and a fairly young structure, such cells are the result of an abnormal maturation with marked discrepancy between the aspect of the cytoplasm and nucleus. In case 2, which later developed a slight neutropenia, a few premyelocytes with irregular nucleus were also seen.

In the 2 patients who exhibited slight toxic changes of the neutrophils in the blood smear, examination of the bone marrow showed (1) in case no. 1, 1 metamyelocyte with coarse granules and 1 immature myelocyte with vacuoles and coarse granules on a total of 500 cells of the leuko- and lymphopoiesis, (2) in case 9, a few neutrophils with slightly coarse granulation, it being impossible to say whether they belonged to the blood or to the marrow.

To complete the picture, we mention that a binucleated cell was seen in 2 cases in no 6, an immature myelocyte with 2 nuclei, in no 9, a neutrophil with 2 rod shaped nuclei with adult structure. This last cell might be a neutrophil myelocyte in which the mitotic division of the nucleus was not followed by a division of the whole cell and where the 2 nuclei continued their maturation, as shown by shape and structure, to the stage typical of adult staff cells.

The number of megakaryocytes was increased in cases no 9 and, to a lesser extent, no 5, which reaction can be seen in some forms of thrombopenia and panmyelopathy.

DISCUSSION

In the cases we have personally observed, we have failed to see the sudden development of severe agranulocytosis, which is largely an allergic manifestation. In 2 cases the leukocyte count dropped below the 3,000 mark, in these, the examination of the bone marrow showed evidence of impaired development* in the granulocyte series: increased percentage of premyelocytes and immature myelocytes, abnormally large premyelocytes, discrepancies in the maturation of nucleus and cytoplasm. It is conceivable that these changes, when allowed to develop further, might eventuate in a toxic agranulocytosis, which, contrary to the acute allergic variety, shows a definite relationship to the amount of toxic substance given.

Erythropoiesis did not suffer, a slight hypochromic anemia in 2 cases improved during treatment.

It should be emphasized that patients treated with thiouracil should have weekly leukocyte counts, together with careful examinations of blood smears with particular reference to the differential count of the white blood cells and to toxic changes in the granulocytes. In some cases, the number of neutrophils may be markedly decreased, although the total number of leukocytes remains within normal limits, and in others, the development of well-defined toxic changes in the neutrophils may be indicative of an impending serious disturbance.

* We purposely avoid the term *maturation* which has been too widely used without discriminating between the true maturation of a cell or group of cells and the ability for mitotic division. (See Rohr pp 107-08.)

STERNAL MARROW STUDIES IN THYROTOXICOSIS TREATED WITH THIOURACIL AND REVIEW OF LITERATURE REGARDING THIOURACIL EFFECTS ON BLOOD

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THE danger of leukopenia or agranulocytosis occurring during thiouracil therapy has been repeatedly emphasized. The purpose of this paper is to present serial studies of peripheral blood and sternal marrow in thyrotoxic patients before and after treatment with thiouracil* and after thyroidectomy.

LITERATURE REGARDING THIOURACIL EFFECT ON BLOOD AND MARROW

A review of the available literature to date shows 1914 cases† of thyrotoxicosis in which thiouracil was employed. This does not include the use of thiourea or other related compounds nor the use of thiouracil in any condition but thyrotoxicosis. Forty-four cases (2.3 per cent) had resulting agranulocytosis, and of these, 15 (34 per cent) died because of the agranulocytosis. Ninety-three patients (4.9 per cent) developed significant leukopenia. There are obvious disadvantages in this method of estimating the occurrence of these complications. However, two recent articles have presented data on the same subject and the results are similar (see table 1). There are unavoidable duplications in these two series and in our review of the literature.

Table 2 shows a brief summary of the 44 reported cases of agranulocytosis due to thiouracil. Eight were male and 26 female (10 unspecified). This ratio probably approximates that of thyrotoxicosis itself. The ages ranged from 16 to 70, the average being 45 years. Marrow findings were described in 15 cases, but in many cases we do not know how long the agranulocytosis had existed when the marrow studies were done. In most cases the marrow was described as hypoplastic, or with maturation arrest. In the granulocyte series, a concept first presented by Fitz-Hugh and Krumbhaar in 1932.²⁴ In only 1 case had a control marrow study been done before thiouracil was given.²⁸

Dameshek^{25a} states that in agranulocytosis the bone marrow goes through the following stages: immediate reaction with reduced numbers of granulocyte precursors, shortly thereafter a loss of mature, then immature granulocytes takes place. If the patient survives, the granulocyte precursors reappear, and this is the stage which has frequently and perhaps erroneously been termed maturation arrest; the mature granulocytes then reappear. Dameshek quotes the work of Plum,^{25b} who performed serial sternal punctures in many of his 114 cases of agranulocytosis, most of them following aminopyrine. Braun²⁶ reports serial

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† Bibliography available upon request.

bone marrow examinations in a case of agranulocytosis apparently due to sulfonamides. There were three sternal punctures in 9 days. The first showed hypoplasia with predominance of lymphocytes. The second showed hyperplasia of young myeloid cells, and no granulocytes were present in the peripheral blood at this time. The third revealed normal marrow with slight myeloid hyperplasia and the peripheral blood showed a leukemoid picture with 33 per cent immature white cells. The results agree with those of Plum.

The mechanism of development of agranulocytosis in thiouracil intoxication is a matter of great interest. Lesses and Gargill¹² reviewed the earlier cases of the use of thiouracil, including 8 cases of agranulocytosis and 47 cases of leukopenia, and stated that "severe or fatal reactions have occurred at dosage levels ranging from 0.2 to 2.0 grams daily after 6 days to 8 months." They concluded that "the evidence is adequate that the dosage-time factor is not a controlling influence in the etiology of thiouracil neutropenia and agranulocytosis." Van Winkle et al.¹ in reviewing 5745 cases felt that these two complications were related to the duration of treatment but not to dosage. Over one-half of their cases of agranulocytosis occurred in the first month. In Moore's 1091 cases² he found the greatest likelihood of agranulocytosis was from the 4th to the 8th week, although the

TABLE 1—Incidence of Agranulocytosis and Leukopenia in Thiouracil Therapy

Author	Total Cases	Agranulocytosis	Mortality in Agranulocytosis	Leukopenia
Van Winkle et al. ¹	5745	5	14	44
Moore ²	1091	174	26	34
Review of Literature	1914	3	24	49

onset might take place at any time from 2 to 36 weeks. Wosika and Braun¹³ reviewed 9 cases of thiouracil-induced agranulocytosis and found 6 were in patients over 50 years of age (2 of unspecified age).

Hypersensitivity is considered an important etiologic factor by many. Fishberg et al.¹⁴ and others have quoted cases in which, after recovery from agranulocytosis, a few doses of thiouracil produced a noticeable reduction in the granulocyte count. Some authors believed that their patients might become desensitized. Thus, in nearly one-half the cases of leukopenia reported by Van Winkle¹ in which the drug was continued the blood count returned to normal. A few cases of agranulocytosis occurred in patients who had had previous courses of thiouracil (4, 11 and 12, our case) and in at least one case the patient had thiouracil without ill effects after recovering from agranulocytosis.¹⁵

Destruction of mature granulocytes is a possible mechanism for the production of agranulocytosis. In the case of Gargill and Lesses (table 2, no. 15) the marrow at autopsy also showed large numbers of disintegrating forms. The case of Lozinski et al. (table 2, no. 29) showed adult neutrophils in the lungs and neurotic tissue of the neck at autopsy 16 days after the onset of agranulocytosis.

The granulocytes are the elements chiefly affected by thiouracil. In the 1914 cases in the literature reference is made to only 2 cases of anemia¹⁶ and to 3 cases

TABLE 2.—44 Reported Cases of Agranulocytosis after Treatment for Thyrotoxicosis

Author	Patients		Reconv. from Agran.	Died of Agran.	Author's Description of Bone Marrow
	Sex	Age			
1 Astwood ^{a, b}	M	37	X		
2 Barr et al. ^c	F	57	X		On 7th day of agran. most cells polymorphocytes with many myeloblasts. Only one P M N cell seen.
3 Barr et al.	M	16	X		
4 Cantor et al. ^d	F	55	X		
5 Drennen ^{e, f}				X	
6 Ferrer et al.	M	70		X	At autopsy mod. hypoplasia granulocytic cells. Def. decr. in C ₁ mature P M N leukocytes.
7 Fishberg et al. ^{g, h}	F	50	X		Maturation arrest in granulocytes.
8 Fishberg et al.			X		
9 Fishberg et al.			X		
10 Fishberg et al.			X		
11 Fishberg et al.	M	28	X		
12 Gabrilove et al. ^{i, j}	F	47		X	(Detailed figures reported. Author's interpretation not stated.)
13 Gabrilove et al.	F	50	X		
14 Gabrilove et al.	F	43	X		
15 Gabrilove et al.	M	58	X		
16 Gabrilove et al.	M	53	X		
17 Gabrilove et al.	F	29	X		
18 Gargill et al. ^{k, l}	F	56		X	2nd and 4th day of agran. hypoplastic marrow with granulocytic aplasia. At autopsy extensive granulocytic hypoplasia.
19 Grainger et al. ^m	F	23	X		
20 Haler ⁿ				X	
21 Himsworth ^o			X		At onset agran. only a few primitive cells. 6 days later, vigorous activity and mature cells.
22 Himsworth				X	
23 Kahn et al. ^p	F	63		X	At autopsy cells of granulopoietic series greatly reduced in number.
24 Lahey et al. ^q	F	36	X		
25 Lahey et al.	F	61	X		
26 Lahey et al.			X		
27 Lahey et al.	F	41		X	
28 Linsell ^r	F	61	X		Marked hypoplasia with arrest of granulocytic elements at myelocyte stage.

TABLE 2.—*Concluded*

Author	Patients		Recov from Agran	Died of Agran	Author's Description of Bone Marrow
	Sex	Age			
29 Lozinski et al ¹⁹	F	49		X	At autopsy myelocyte arrest Deer in myeloid series
30 McGavock et al ²⁰	F	50	X		
31 Meyer, A H ¹	M	40	X		Deer total myeloid cell count and arrest of maturation of myeloid series
32 Pearson ²²	F	32	X		
33 Rothendler et al ²³	F	52	X		
34 Rubinstein ⁵	F	47	X		
35 Simpson ²⁶			X		At autopsy mud hypoplasia with marked deer in mye loid elements
36 Trasoff et al ⁷	M	34		X	
37 Tyson et al ⁸	F	49		X	Hypoplastic Disappearance of all mature and most of im mature granulocytes At autopsy similar
38 Tyson et al	F	59		X	3 myeloblasts 1 premyelocyte No older granulocytes
39 Tyson et al	F	47		X	Marked deer in myeloid cells No mature granulocytes
40 Tyson et al	F	32		X	Few premyelocytes and myelo cytes No mature granuloc ytes (To be described below)
41 Williams et al ^{9 30 31}	F		X		
42 Williams et al	F		X		
43 Wosika et al ²²	F	50		X	
44 Our patient†	F	20	X —	—	
Totals			29	15	

* These cases also reported by Tyson et al ²⁸ with other cases

† Previously reported by Carns and Poser ²²

of purpura or of thrombopenia,^{4 13 35} and some of these were regarded as unrelated to the therapy. Williams showed that the bone marrow takes out a relatively high concentration of thiouracil.^{39 40} Palmer⁴¹ gave large doses of thiouracil to patients dying of nonthyroid conditions and found high concentration of thiouracil in the adrenals, bone marrow, and pituitary gland at autopsy. Fishberg and Vorzimer,⁶ however, were unable to demonstrate thiouracil in the bone marrow aspirate of a case of agranulocytosis which recovered. This may have been due to its rapid excretion⁴⁰, the exact time relations are not stated. Warren⁴ studied the respiratory metabolism of rabbit femoral marrow and exudates *in vitro* and concluded that levels of thiouracil comparable to those in the bone marrow of patients on therapeutic doses induce a small but significant inhibition of respiration of rabbit bone marrow cells *in vitro*, the effect on the myeloid elements and especially on the immature myeloid cells being more striking than on others. Attempts

to oppose the action of thiouracil with pyridoxine were unsuccessful. Cantor and Scott⁵ suggest that pyridoxine may prove to be the granulocyte maturation factor. Fishberg and Vorzimer⁹ postulated that pyridoxine, because it caused such rapid improvement in their cases, must act on the mechanism of release of granulocytes rather than on their maturation. Paschke et al.¹² showed that 0.002 M solution of thiouracil inhibits significantly the cytochrome oxidase activity of thyroid tissue *in vitro*, but that this enzymatic activity of bone marrow could not be inhibited *in vitro* by 0.002 to 0.01 M concentration of thiouracil. The nature of this protective mechanism is unknown, but some work tends to show that this protection can be overcome by higher concentrations of thiouracil, thus again raising the question of the importance of the dosage-time factor in development of agranulocytosis. Reveno¹⁴ records a case of a patient who had received thiouracil for 381 days and suddenly died of cerebral hemorrhage. The bone marrow at post-mortem was hyperplastic for an individual of this age (female, 74, but no other details are given).

EFFECT OF THYROTOXICOSIS ON BLOOD PICTURE

The literature regarding the effect of thyrotoxicosis itself on the blood picture was reviewed rather extensively in 1932 by McCullagh and Dunlap,⁴³ again in the same year by Hertz and Lerman,⁴⁶ and in 1940 by Woodruff.⁴⁷ Plummer³ in 1919 studied 578 cases and reported an average leukocyte count of 6,793 per cu mm. He felt that the blood picture was of little aid in diagnosis or for judging the severity of the disease. Menkin⁴⁹ found a relative lymphocytosis (over 30 per cent in 67 of 100 cases) and believed it was produced by way of the sympathetic nervous system. He demonstrated lymphocytosis in cats under excitement, this did not result if the animal had had a previous sympathectomy or splenectomy. Gortlieb's work⁵¹ tended to show that additional lymphocytes might be added to the blood from the toxic thyroid gland. In 11 cases studied at operation, the average lymphocyte count in the thyroid artery was 44 per cent and in the thyroid vein 50 per cent. McCullagh and Dunlap,⁴⁵ having studied 1200 cases, stated that the total leukocyte count varied more widely in thyrotoxic than in normal patients. They believed that this was chiefly due to a variation in the numbers of granulocytes. They found an absolute increase in lymphocytes. In averaging 250 differential counts they found an absolute increase in lymphocytes, and in 60 per cent of 266 cases a relative lymphocytosis of 30 per cent. They considered the relative lymphocytosis of diagnostic but not of prognostic value. Jones⁶² found myeloid hyperplasia in the marrow of 700 thyrotoxic patients. It is said⁴⁶⁻⁴⁷ that the red cell count and hemoglobin are normal or slightly reduced. In 1944 Conklin and Shrank⁶³ reported a case of thrombocytopenic purpura and found five similar cases in the literature.

Little has been written as to the effect of iodine on the blood picture in thyrotoxicosis. Jackson⁶⁴ says it has none, while Hertz and Lerman⁴⁶ found changes, primarily a decrease in monocytes.

The present study was made on 27 patients from the Wisconsin General Hospital. All had definite thyrotoxicosis and, except as mentioned, had no complications.

which might be expected to alter the blood picture. One series (9 patients) received thiouracil and the control series (20 patients) received iodine. A few patients appear in both series, having been given thiouracil after iodine failed to secure a remission. Whenever possible observations were made before any treatment was given, but some patients had received iodine some time before entering the hospital. Our thiouracil series overlaps that of Carns and Poser.²³

METHODS

In so far as possible each patient received blood counts and sternal marrow study before treatment, at the end of the course of thiouracil or iodine, and about one week after surgery. The hematologic work-up was performed by the same person (E. W. T.) in all cases. The pipets and counting chamber were standardized by the U. S. Bureau of Standards. For both peripheral blood and marrow aspirates, cover slip preparations were made and stained with Kingsley stain. The blood counts included hematocrit determinations using Wintrobe tubes, reticulocyte

RESULTS

TABLE 3—Peripheral Blood Counts—Normal Findings²⁴

	Thousands Leukocytes	% Neutro	Thousands Abso Neutro	% Eos	% Bas	% Lymph	Thousands Abso Lymph	% Mono	% Retic
Min	5.0	57.0	3.15	1.0	0.0	25.0	1.5	3.0	0.5
Mean	7.0		4.3				2.1		
Max	10.0	57.0	6.2	3.0	0.75	33.0	3.0	7.0	1.5

counts, and a differential count of 500 white cells. The number of platelets was estimated from study of the stained films and classified as normal, increased, or decreased. We consider this more reliable than most platelet counts. In cases receiving thiouracil, blood counts were also performed three times weekly during the period of therapy.

The standard thiouracil dosage was 0.2 Gm. three times a day. Toward the end of the course this was changed to 0.4 Gm. three times a day in 2 cases and to 0.1 Gm. three times a day in 2 cases. The iodine-treated cases were given Lugol's solution, 5 to 10 drops three times a day except for 2, who received 1 minim of sodium iodide daily. If previous iodine therapy had ended at least one month before our studies, it was assumed there was no residual result on the blood picture. If the interval were less, this has been noted in tables 4 and 6 presenting our results, where we have also noted the few cases in which iodine was added to the thiouracil therapy shortly before surgery. The surgical procedure in each case consisted of a one stage subtotal thyroidectomy. Surgery was not done in 3 cases treated with thiouracil and in 1 case prepared with iodine (toxic psychosis).

Control peripheral blood counts in thyrotoxic patients (table 4) as compared with normal counts (table 3) show a wider range of values, but the mean counts are comparable in each case except for the percentage of monocytes, which is in

TABLE 4—*Peripheral Blood Counts in Cases of Thyrotoxicosis*

	Days of Treatment	Days P O	Thousands Leukocytes	% Neutro	Thousands Abso Neutro	% Eos	% Bas	% Lymph	Thousands Abso Lymph	% Mono	% Retic	Platelets
19 Control Counts*												
Min			4.2	42.2	2.1	0.2	0.0	12.8	0.9	4.6	0.0	0 decr
Mean			7.4	60.6	4.6	1.9	0.2	27.2	2.0	9.0	0.5	9 normal
Max			14.0†	76.4	8.6	5.0	1.0	49.2	3.6	19.2	1.6	10 incr
19 Cases after Iodine												
Min	7.0		4.0	34.8	1.4	0.2	0.0	12.2	1.3	2.8	0.0	1 decr
Mean	51.4		7.4	58.9	4.6	2.1	0.2	29.4	2.0	7.8	0.4	15 normal
Max	240.0		16.0†	81.8	12.2	6.6	1.0	53.0	3.1	16.4	1.3	3 incr
9 Cases after Iodine and Thyroidectomy												
Min	7.0	5.0	4.5	52.6	2.4	0.6	0.0	9.8	0.8	3.6	0.2	0 decr
Mean	12.8	7.5	8.1	64.5	5.4	2.7	0.2	25.0	1.9	7.0	0.7	5 normal
Max	17.0	10.0	12.1†	79.8	8.8	4.4	1.0	36.2	2.7	11.6	1.0	4 incr
10 Cases after Thiouracil‡												
Min	9.0		3.6	31.0	1.1	0.0	0.0	19.0	1.2	2.4	0.1	0 decr
Mean	25.9		7.6	61.4	4.9	1.8	0.2	28.0	2.0	8.3	0.5	8 normal
Max	84.0		11.0†	75.4	7.5	3.4	0.8	50.0	3.1	15.6	1.6	2 incr
5 Cases after Thiouracil and Thyroidectomy§												
Min	25.0	6.0	4.7	62.2	2.9	0.0	0.0	19.2	1.2	6.8	0.2	0 decr
Mean	36.2	6.8	8.2	65.1	5.3	0.6	0.1	24.7	2.0	9.0	1.1	1 normal
Max	52.0	8.0	9.8	70.4	6.9	1.6	0.2	29.4	2.7	11.6	2.3	4 incr

Abbreviations Days P O—days postoperative Abso Neutro—absolute neutrophil count, Eos—eosinophils Bas—basophils Abso Lymph—absolute lymphocyte count Mono—monocytes Retic—reticulocytes

* Five cases had iodine for 2 to 3 days and 2 cases had thiouracil for 1 and 2 days just before these studies

† These cases showed no apparent infection

‡ This does not include the agranulocytosis case but does include the patient with severe neutropenia Six cases had previous iodine therapy (for 3, 14, 60, 90 and 240 days respectively) in one case ending 15 days before these studies but in all others ending 24–30 days before them Three cases had iodine added just before surgery (for 1, 3, and 6 days respectively)

§ Two cases had iodine added to therapy immediately preoperatively and received it for 7 and 10 days (ending 6 and 7 days before these studies)

creased, platelets are normal to increased in number. Counts after iodine as compared with the control observations show a slight decrease in monocyte percentage, but not back to the normal level, platelets have returned to normal. After thiouracil there is no apparent change from the control counts, except that the

TABLE 5—Sternal Marrow Studies Normal Values³³

	% Neutro	% Eos	% Bas	% Lymph	% Mono	% Meta	% Myelo	% Blasts	% Premy	% Normo
Min	7 0	0 5	0 0	3 0	0 5	13 0	5 5	0 3	1 0	~ 0
Mean	20 0	2 0	0 2	10 0	2 0	22 0	13 9	2 0	5 0	18 0
Max	30 0	4 0	0 7	17 0	5 0	32 0	2~ 5	5 0	9 0	3~ 0

TABLE 6—Sternal Marrow Studies in Cases of Thyrotoxicosis

	Days of Treatment	Days P O	% Neutro	% Eos	% Bas	% Lymph	% Mono	% Meta	% Myelo	% Blasts	% Misc	Normo Leuk	Platelets
18 Control Counts*													
Min			31 2	0 4	0 0	12 0	0 2	6 6	3 0	0 6	1 4	20 0	6 decr
Mean			43 8	1 6	0 0	22 4	1 6	9 7	14 0	2 2	3 9	59 0	11 normal
Max			55 6	2 8	0 0	31 4	4 8	12 0	23 8	5 2	7 2	109 0	1 incr
20 Cases after Iodine													
Min	7 0		22 6	0 6	0 0	13 2	0 0	5 6	9 4	0 4	0 4	32 0	6 decr
Mean	51 9		41 1	1 4	0 0	25 3	1 0	10 7	14 7	2 2	3 7	58 9	14 normal
Max	240 0		61 6	2 6	0 2	40 8	3 2	16 0	21 8	5 0	9 6	132 0	0 incr
9 Cases after Iodine and Thyroidectomy													
Min	7 0	1 0†	36 2	0 8	0 0	14 0	0 8	5 0	5 2	0 4	0 2	10 0	0 decr
Mean	13 4	6 7	46 5	2 7	0 0	21 4	2 5	10 8	13 7	1 4	1 7	34 6	8 normal
Max	17 0	10 0	62 4	3 0	0 2	31 2	5 6	16 8	23 8	2 4	4 4	75 0	1 incr
10 Cases after Thiouracil‡													
Min	9 0		30 4	0 0	0 0	19 4	0 8	4 0	0 6§	0 4	0 2	15 0	2 decr
Mean	25 9		46 1	1 4	0 0	25 8	2 0	9 2	10 7	1 7	3 1	56 7	7 normal
Max	84 0		60 0	3 0	0 4	43 0	7 0	16 0	24 0	4 0	6 6	95 0	1 incr
4 Cases after Thiouracil and Thyroidectomy													
Min	25 0	6 0	37 8	0 0	0 0	14 0	1 4	9 2	4 2	1 0	0 6	35 0	2 decr
Mean	38 0	7 8	47 1	0 5	0 0	19 2	2 4	12 0	13 0	1 7	3 0	57 3	2 normal
Max	52 0	11 0	56 2	1 0	0 0	28 2	3 6	18 6	23 0	2 4	5 0	74 0	0 incr

Abbreviations as in tables 3 and 4, Meta—metamyelocytes, Myelo—myelocytes of all types including promyelocytes also in table 4, Premy—promyelocytes, Normo—normoblasts Misc—miscellaneous types of cells, including large young, endothelial pathological, primitive plasma disintegrating and unclassified cells

* ‡ As in tables 3 and 4.

† The next shortest interval after surgery was 5 days

§ This is the case of severe neutropenia to be discussed later

platelets have returned to normal. The two groups of postoperative studies show similar changes when compared with the appropriate preoperative counts—a slight

increase in leukocyte count, in relative and total neutrophil count, and in reticulocytes, lymphocytes show a slight relative decrease. Platelets are again normal to increased. These are the findings commonly described after surgery of any type. The increase in reticulocyte count after thiouracil and surgery is greater than the increase after iodine and surgery, and this difference may be a significant one.

The results of the sternal marrow studies are presented in table 6. Our control counts are dissimilar in some ways to the normal values which Wintrobe records (see table 5). However, it is well known that a variety of methods are employed in sternal marrow studies and that results vary widely. It is not likely that this is a profitable comparison. The counts after iodine are similar to the control counts. After thiouracil there tends to be a slight increase in neutrophils and slight decrease in myelocytes as compared with control counts. This is probably not significant. The postsurgical counts show no marked changes. The two groups (each compared with the appropriate presurgical group) show decrease in lymphocytes and increase in leukocytes (adult or young). These are similar to the changes already described in the peripheral blood after operation.

As already mentioned, patients receiving thiouracil had blood counts three times a week. The counts after thiouracil reported in table 4 include only those done at the end of therapy. A review of the additional counts (except for the two severe reactions with case reports below) shows that in 5 of 7 cases nothing unusual appeared in these counts, and specifically the leukocytes did not fall as low as 5,000. In 1 case a single leukocyte count was 4,800 (with 72 per cent neutrophils) but thiouracil was continued and no symptoms or further leukopenia developed. In one woman of 56 the admission count was 5,550 leukocytes with 39 per cent neutrophils, and the sternal puncture showed 30.0 per cent neutrophils. Thiouracil was then given for 9 days with white counts ranging from 3,300 to 5,200, with neutrophils from 28.8 to 39.2 per cent. Sternal puncture at the end of this time showed 30.4 per cent neutrophils, the lowest of that group in table 4. She had no unusual symptoms. Thiouracil was stopped because of the persistent moderate leukopenia and neutropenia, and Lugol's solution was used for 20 days. The white counts continued to vary from 3,700 to 6,100, with neutrophils from 29.4 to 41.0 per cent. The counts are similar before treatment, after thiouracil, and after iodine and therefore cannot be regarded as the result of the thiouracil.

Reviewing all counts on thiouracil-treated patients with special attention to the hemoglobin and erythrocyte counts, we found that 7 of 9 patients had higher hemoglobin and 5 of 9 patients had increased red cell counts after thiouracil. The changes in either direction were slight. No cases of anemia developed. One case of anemia of unknown cause was present at admission and unchanged during thiouracil administration.

In our small thiouracil series of 9 patients, 1 case of agranulocytosis and 1 case of severe neutropenia occurred. Following is the report of the case of agranulocytosis.

E. W., a 20 year old woman, was admitted to the Wisconsin General Hospital on 8-2-44 with history and findings of thyrotoxicosis of the Graves type which began one and one half years before and was obviously severe at admission. Previous treatment had included iodine for 2-3 months beginning June

1943 and again from 3-19-44 to 3-30-44 with thiouracil 0.2 Gm. three times a day from 3-5-44 to 4-19-44 when she left the hospital because of social problems. She did not reconsult her physician until June 1944 when thiouracil was again employed for 1 month. She had had no treatment for 1 month before admission 8-2-44.

The thyroid was large and smooth and the classical findings of severe thyrotoxicosis were present. The basal metabolic rate was +91 at admission. The patient was about 7 months pregnant. There was no apparent response to the administration of 2 minims of sodium iodide twice daily for 18 days. On

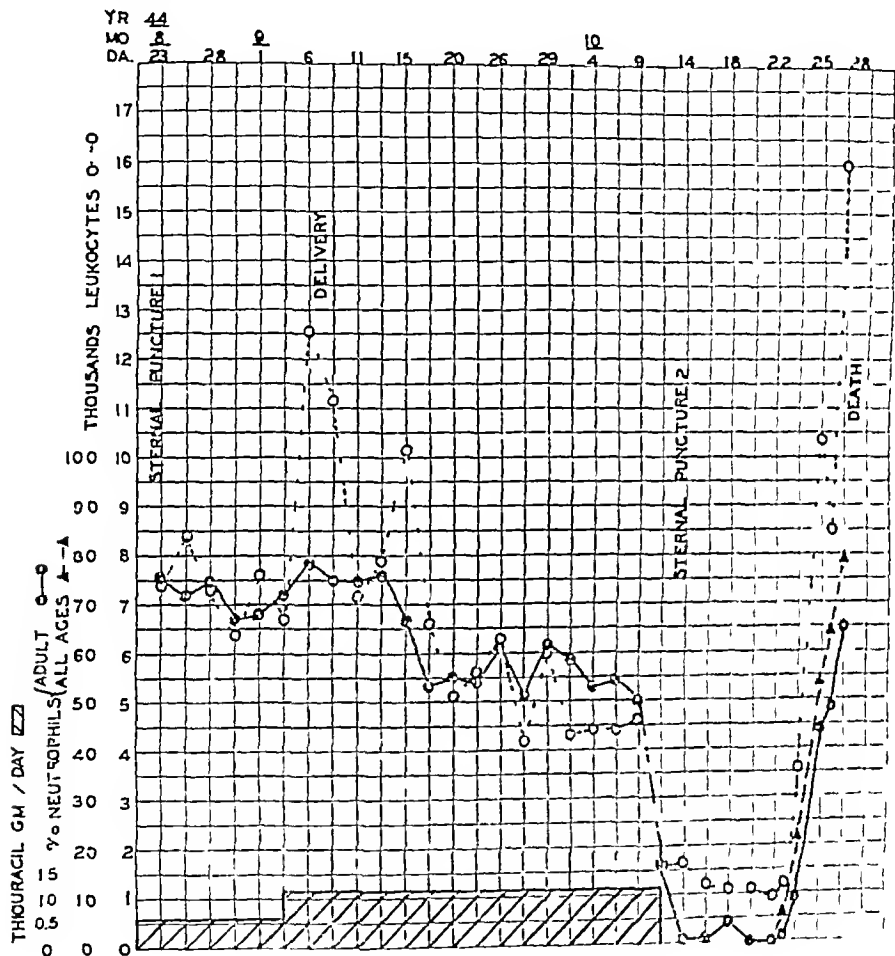


FIG. 1. Case of Agranulocytosis from Thiouracil

August 22, 1944, thiouracil 0.2 Gm. every eight hours was begun. Basal metabolic rate varied from +48 to +125. On September 2, thiouracil dosage was advanced to 0.4 Gm. every eight hours. Spontaneous premature delivery of a healthy male child occurred on September 7, 1944. Beginning about September 20, it was observed that the leukocyte count, which had previously been 6,000 or more, had dropped to about 5,000, but the neutrophil count continued to be slightly above 50 per cent, as it had been from the beginning (see fig. 1).

On October 13, after the previous borderline counts, leukocytes were 1,600 with 16 per cent neutrophils. There were no symptoms of agranulocytosis until the following morning when she had a temperature

ture of 102 degrees and her throat was sore, edematous, and red. Thiouracil had been stopped on October 13 and the following treatment started: (1) penicillin 15,000 units every 4 hours intramuscularly, (2) folic

TABLE 7—Sternal Punctures in Case of Agranulocytosis

Date 1944	Days of Iodine	Days of Thiouracil	% Neutro	% Tos	% Bas	% Lymph	% Mono	% Metr	% Myelo	% Blast	% Mice	Normo/400 leuk	Platelets
8-23	18	1	51.6	0.4	0.0	12.0	3.0	11.0	17.8	1.4	2.8	45	normal
10-14		54	0.0	0.0	0.0	84.8	0.4	0.0	0.0	9.2	5.6	442	decr
10-28		54	9.0	0.0	0.0	24.0	0.0	21.2	39.0	0.8	6.0	10	"

TABLE 8—Case of Severe Neutropenia from Thiouracil

Date 1945	Treatment	Thousands Leuko	% Neutro	% Eos	Comment
5-11		10.7	81.8	0.2	Sternal Puncture #1
5-12					Thrombophlebitis diagnosed
5-16	TU 0.2 Gm tid started				T 101.9
5-17		7.9	66.0	3.8	
5-21		4.3	43.0	3.6	Afebrile from here on
5-23		8.0	53.8	4.4	
5-25		6.6	59.2	3.2	
5-28		5.0	40.8	3.8	
5-31	TU stopped	3.0	54.0	3.2	Abd pain and vomiting No fever Throat negative
6-1		4.8	41.4	2.0	
6-4		4.8	49.4	2.8	
6-6	TU 0.1 Gm bid started				
6-8		3.9	42.8	6.2	
6-11		4.7	44.8	3.4	
6-12					Sternal puncture #2
6-14	Thyroidectomy TU stopped				Surgery without incident No fever
6-15					T 102.4
6-17					T 104.2
6-18	Penicillin 20,000 U q 3 hrs Pentnucleotide 10 cc IM bid started 500 cc whole blood I V	1.7	18.0		T 103.2
6-19	500 cc whole blood I V	4.8	41.0		T 101
6-20	Pentnucleotide stopped	4.7	62.2	0.0	T 100.2
6-23	Penicillin stopped				Afebrile from 6-21
6-25					Sternal puncture #3
6-26		4.9	64.0		
6-30		6.7	67.0		Condition satisfactory Discharged

acid 4 capsules five times a day (3) crude liver extract 6 units daily intramuscularly (4) pentnucleotide 20 cc intramuscularly twice a day, and (5) two 250 cc transfusions of fresh whole blood. The folic acid was later stopped because of nausea and was replaced by yellow bone marrow extract 4 cc three times

daily orally. She continued essentially without neutrophils until October 23 when she had 1 adult form and 5 young forms. Her further recovery from the agranulocytosis is best seen in fig. 1. The clinical course from October 14 on showed temperature of 104-105 degrees, pulse 140-160 and the throat continued edematous and the gums fiery red with some areas of ulceration.

By October 25, 1944, the agranulocytosis was apparently cured but the thyroid toxicity became a major problem. On October 26 and October 27 she was given x ray therapy (100 r) to the thyroid gland. By October 27 the signs of thyroid crisis were definite: temperature 107 degrees (rectally), pulse irregular rate 160, diarrhea. She was treated with intravenous Thevetin¹⁴ and general supportive measures but the pulse reached as high as 230 and she died on October 28, 1944.

At autopsy, 7 hours after death, the microscopic examination showed myelocytic hyperplasia of the vertebral bone marrow and an aplastic femoral marrow. Sternal marrow studies were done with technic similar to that in previous examinations (see table 7). This case has been previously reported by Carns and Poser.¹⁵

The following case report illustrates severe neutropenia following thiouracil therapy.

E. B., a 54 year old Italian woman, was admitted on May 9, 1945, with a 1 year history typical for thyrotoxicosis. She had received Lugol's solution, 10 drops three times a day, for 8 months, up to the time of admission. Physical findings were those of a thyrotoxic individual with a nodular goiter with arteriosclerotic and hypertensive heart disease with cardiac enlargement, auricular fibrillation and functional capacity II. Admission basal metabolic rate was +76 undoubtedly inaccurate because of

TABLE 9—Sternal Punctures in Case of Severe Neutropenia

Days of Iodine	Days of Thiouracil	Days P O	% Neutro	% Eos	% Bas	% Lymph	% Mono	% Meta	% Myelo	% Blasts	% Misc	Normo / 500 Leuk	Platelets
240	0		61.6	1.0	0.0	13.2	1.2	9.0	9.4	1.2	3.4	32	Normal
	21		49.4	1.2	0.0	33.8	1.4	6.2	0.6	3.0	4.4	71	Deer
	22	11	56.2	0.0	0.0	21.2	3.6	10.0	4.2	1.4	3.4	59	Deer

language difficulties and the patient's apprehension. Blood count on 5-11-45 showed 14.0 grams hemoglobin, 4,900,000 red blood cells, 10,700 white cells with 81.8 per cent neutrophils.

The progress of the case is seen by reference to table 8. The sternal puncture findings are in table 9. The second sternal puncture was done routinely at the end of thiouracil therapy and not because the patient had any new signs or symptoms. It shows a decrease in young and adult members of the granulocyte series with return to normal in the third sternal puncture.

DISCUSSION

We have demonstrated no significant changes which occur regularly in the blood or marrow of patients receiving thiouracil. In certain relatively unpredictable cases leukopenia or agranulocytosis occurs. It is probably significant that our 2 cases of severe reaction followed periods of borderline leukopenia. Although many patients with such counts do not progress to a serious reaction we believe it advisable for all patients to have leukocyte and differential counts three times weekly during the entire course of thiouracil administration regardless of its duration, and to have these counts repeated daily if values are borderline or symptoms develop.

Analysis of the blood and marrow findings in our 2 cases of severe reaction shows they are consistent with the series of events described by Dameshek,^{15a} Plum^{15b} and Braun.¹⁶ In our case of agranulocytosis the blood and marrow studies were normal at the beginning of thiouracil treatment. On the day preceding symptoms the patient had 1,600 leukocytes with 16 per cent neutrophils, and on the

first day of symptoms the marrow showed a complete absence of young and adult neutrophils (as was the case in the peripheral blood) It is impossible to state whether the 92 per cent blasts were chiefly myeloblasts or lymphoblasts In the first few days of beginning recovery, the peripheral blood showed more young than adult neutrophils Terminally she had 16,000 leukocytes with 78.5 per cent total neutrophils, and the final marrow examination (14 days after the previous one) revealed a reduction of the percentage of blasts to the normal range, but the increase then was chiefly in the myelocytes and metamyelocytes with only a slight return of the adult neutrophils by that date

In the case with neutropenia, the original blood and marrow observations were within normal range The second marrow study was done 3 days before fever began—the first clinical indication which was probably dependent upon the neutropenia The marrow showed definite reduction in myelocytes, and the metamyelocytes were few By the time of the third sternal puncture, the patient had recovered clinically and the values were back to normal In both cases the sequence of events is more readily correlated with the concepts of Dameshek³⁵ than with the relatively prevalent idea of maturation arrest at the myelocyte stage

Our cases do not add much to the understanding of the mechanism of development of agranulocytosis In our case with agranulocytosis we cannot rule out the possible importance of the dosage-time factor, hypersensitivity seems quite unlikely In the case with neutropenia the reverse is true (note eosinophil column in table 8) It may be that all cases do not develop by the same mechanism

Our series is too small for definite conclusions, but in some respects the results tend to confirm previous observations of others, such as the following Thiouracil affects chiefly the neutrophils and particularly the younger ones Agranulocytosis begins with alarming suddenness The leukocyte and neutrophil counts vary more widely in thyrotoxic patients than in normal people When thyrotoxicosis is treated with iodine the chief change in the blood picture is a reduction in the percentage of monocytes We do not know of previous reports of the effect of iodine on the bone marrow We found no changes attributable to this treatment

A word might be said about the incidence and mortality of agranulocytosis from thiouracil A 2 per cent incidence of this complication with about 25 per cent mortality means 1 death in each 200 cases treated It is possible that this figure may be reduced with the use of penicillin^{24, 28} and possibly pyridoxine^{5, 9, *}

CONCLUSIONS

- 1 Serial sternal marrow and blood studies fail to show any regularly occurring changes after thiouracil therapy Changes are seen only in the few relatively unpredictable cases in which leukopenia or agranulocytosis develops

- 2 A review of the literature indicates that the incidence of agranulocytosis after thiouracil is about 2 per cent and its mortality about 25 per cent with present forms of treatment

- 3 Our studies support the view that agranulocytosis from thiouracil consists of an extreme reduction of granulocyte precursors and later of the more mature

*E B Astwood and W P Van der Laan of Boston (personal communication) have treated 130 consecutive cases of hyperthyroidism with *propyl* thiouracil and have noted no reactions of a general or hematologic type —Editor

forms with recovery in the same order. They do not support the concept of maturation arrest at the myelocyte stage.

4 Possible mechanisms for the development of agranulocytosis after thiouracil are discussed.

5 The effects of thyrotoxicosis itself on the blood and marrow are reviewed.

6 Two case reports are presented of severe reactions to thiouracil: one of agranulocytosis and one of severe neutropenia.

7 Patients receiving thiouracil should have frequent leukocyte and differential counts. It is recommended that these be performed at least three times weekly and daily if the leukocytic values are suggestive of granulocytopenia.

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THE EFFECT OF THIOURACIL ON LEUKEMIA*

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ONE of the toxic reactions of therapeutic doses of thiouracil† in the treatment of hyperthyroidism is the development of granulocytopenia. A nonfatal granulocytopenia and a fatal type of agranulocytosis have been reported. The selective action of thiouracil on the granulopoietic tissue is the basis for the present evaluation of the drug in the treatment of leukemia.

The 6 cases studied included 4 of chronic myeloid leukemia, 1 of chronic lymphatic leukemia, and 1 of acute myeloid leukemia.

Hematologic surveys, including sternal marrow examination, were made in each case prior to and at frequent intervals during and after the administration of thiouracil. The method used for obtaining and studying the marrow is described by Limarzi.¹ The basal metabolic rate and certain blood chemical constituents including uric acid and cholesterol were determined before, during, and following treatment with thiouracil. Creatine balance studies were carried out in 1 case of acute myeloid leukemia during the administration of the drug.

It will be noted in table 1 that the total amount of thiouracil given varied from 1.6 Gm. administered over a period of 8 days to 274 Gm. administered in gradually increasing doses over a period of 3 months. The average daily dose ranged from 0.2 Gm. to 3.0 Gm. Thiouracil was without effect on the basal metabolic rate, and the blood chemical findings were not appreciably changed except in case 5. In the patient (case 5) with chronic myeloid leukemia, who developed an extreme neutropenic leukopenia following treatment with thiouracil, the blood uric acid gradually rose to 11.4 mg. per 100 cc. of blood prior to death.

The blood picture and bone marrow (table 2), except in case 5, failed to show any marked change after the administration of thiouracil.

In a 7 year old boy (case 1)‡ with acute myeloid leukemia who received approximately 0.3 Gm. of thiouracil daily for a period of 1 month, there were no changes either in the blood picture or the creatine metabolism.

A case of chronic myeloid leukemia with specific cutaneous lesions (case 2) received a daily dose of 0.2 Gm. of thiouracil for a period of 8 days. The rapid terminal phase with an acute blood pattern (hiatus leukemicus) was similar.

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† The thiouracil was supplied through the courtesy of Dr. George R. Hazel of the Abbott Laboratories, North Chicago, Illinois.

‡ This case was studied by Dr. H. G. Poncher of the Department of Pediatrics.

to that observed by Paul and Limarzi² in a case of myeloid leukemia which had not received thiouracil. At autopsy the hemopoietic, as well as the other organs, showed a general infiltration of tissue by myelogenous cells of all types. There was no morphologic thyroid hyperplasia.

A woman with chronic myeloid leukemia (case 3) was given thiouracil in dosage of 1.7 Gm daily for 11 days. The drug was well tolerated. Shortly after the drug was discontinued, the patient developed some gingival oozing of blood. The oral bleeding increased with the addition of retinal and conjunctival hemorrhages. Examination of the blood revealed a marked reduction in the platelet count and an increased bleeding time. The bleeding continued and the patient died following

TABLE 1—*Cases of Leukemia Treated with Thiouracil*

Sex and Age	Type of Leukemia	Total Amount of Thiouracil	Days on Thiouracil	Average Daily Dose of Thiouracil	Blood Chemistry (mg per 100 cc of blood)		Basal Metabolic Rate
					Uric Acid	Cholesterol	
Case 1 Male, 7 yrs	Acute myeloid	Gm 10.0	30	Gm 0.33	3.9 3.3	157 167	+12 +8
Case 2 Female, 46 yrs	Chronic myeloid (with specific cutaneous lesions)	1.6	8	0.2	1.9 4.8	151 160	+56 +36
Case 3 Female, 23 yrs	Chronic myeloid	18.8	11	1.7	1.8	190	+29
Case 4 Male, 25 yrs	Chronic myeloid	80.0	100	0.8	3.8 4.0	200	+23
Case 5 Female, 50 yrs	Chronic myeloid	274.0	90	3.0	3.9 11.4	224	+44 to +77 +36
Case 6 Male, 53 yrs	Chronic lymphatic	60.0	75	0.9	4.1 4.6		+33 +10

a cerebral hemorrhage. This patient's white cell count fluctuated from day to day during the administration of thiouracil, and 48 hours prior to death the white cell count was estimated at 510,000. The blood and bone marrow examination failed to show any morphologic changes of the granulopoietic elements that could be attributed to the toxic effect of the drug. It is well known that the leukocyte count in cases of leukemia is subject to daily fluctuation³ and a marked increase in the white cell count at the time of death is known to occur in leukemia. For this reason it is difficult to say to what extent the drug affected the case studied.

A case of chronic myeloid leukemia in a 25 year old male (case 4) with a white cell count of 370,000 was first given a series of roentgen treatments over the spleen

TABLE 2.—Blood and Bone Marrow Findings in Cases of Leukemia Before and After the Administration of Thiouracil

Peripheral Blood													Bone Marrow											
Case	Hemoglobin Gm 100 cc.	Erythrocytes Mill.	Leucocytes Thous.	Hematocrit % (Red)	Hematocrit % (White)	Sedimentation Rate (Westergren) mm / hr	Jensen Index (units)	Reticulocytes %	Mean Corp Volume cu m	Mean Corp Hb gm cm	Mean Corp Conc. %	Percentage								Platelets Thous.	Remarks			
												Plasma and erythrocytes	Neutrophils %	Metamyelocytes	Band Forms	Poly-nuclear	Eosinophils	Basophils	Monocytes			Lymphocytes	Normoblasts	
Case 1	9.0	2.5	21.0	26.0	1.0	65	7.5	0.5	101	36	31	60	1.0	1.0	0	11	3	0	10	14	3	Dec	Hyperplastic with blast cells	
	8.5	3.2	93.0	29.0	3.0	65	5.0	1.0	90	26	29	82	0.0	1.0	0	6	4.0	0	3	4	0	Dec	Markedly blastic marrow	
Case 2	8.5	3.19	18.7	27.0	2.0	45	10.0	0.5	84	27	32	17	15.0	9.0	3	26	2	2	16	10	0	Dec	Hyperplastic with mod blast' cells	
	3.0	85	60.0	8.0	5.0	70	5.0	0.5	90	35	37	62	6	3.0	0	12	2.0	1.0	4	10	0	Dec	Markedly blastic marrow	
Case 3	7.5	2.47	255.0	25.0	15.0	59	5.0	0.5	101	30	30	15	21	11.0	13	25	3.0	5.0	0.0	4	7	Dec	Hyperplastic—myeloid type	
	11.5	5.50	510.0	32.5	25.0	27	2.0	0.5	92	32	35	28	18	36.0	5	20	2.0	3.0	0.0	7	0	Dec	Hyperplastic—myeloid type	
Case 4	5.5	2.28	90.0	15.0	20	65	4	7.5	0.1	70	24	36	0	18	47	7	20	4.0	6.0	2.0	1.0	3	Nor	Hyperplastic—myeloid type
	10.0	5.07	300.0	26	7.0	15	2.0	1.0	91	32	36	1.0	17	37	0	27	6.0	9.0	1.0	2.0	0	Nor	Hyperplastic—myeloid type	
Case 5	10.0	4.00	410.0	11	21	40	5.0	0.8	77	25	32	2	39	32	2	12	7	2	2	2	0	Nor	Hyperplastic—myeloid type	
	0.0	4.35	1.0	38	trace	27	13	1.5	89	20	21	18.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	49	0	Dec	Celaithous & granulopoietic hypo plasma	
Case 6	5.5	1.89	170.0	17.0	7.0	25	7.0	7.5	0.5	90	30	0.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0	98	0	Nor	Lymphoid infiltration	
	6.0	2.06	130.0	18.0	5.0	15	17.0	5.0	0.5	29	33	0.0	0.0	0.0	0.0	6.0	0.0	0.0	4.09	90	0	Dec	Lymphoid infiltration	

The leukocyte count gradually dropped to 12,100, the splenomegaly disappeared, and the clinical condition became improved. The differential blood smear revealed

TABLE 3—*Leukocyte and Differential Blood Counts in a Case of Myeloid Leukemia Who Received 3.0 Gm of Thiouracil Daily*

Percentage												
Date	Leukocytes	Hematocrit (white cell %)	Myeloblasts	Neutrophilic Myelocytes	Neutrophilic Metamyelocytes	Band forms	Polymorphonuclear Neutrophils	Eosinophils	Basophils	Monocytes	Lymphocytes	Remarks
1945												
2-3	450,000											Before thiouracil
2-7	440,000	24.0	2.0	39.0	32.0	2.0	12.0	7.0	2.0	2.0	2.0	Thiouracil started
2-8	445,000											
2-13	410,000											
2-15	380,000											
2-26	180,000			11.0	17.0	48.0	49.0	1.0	1.0	0.0	21.0	
2-27	230,000											
2-28	290,000	12.0		14.0	22.0	49.0	49.0	2.0	0.0	0.0	13.0	
3-12	290,000	12.0		14.0	22.0	49.0	49.0	2.0	0.0	0.0	13.0	
3-22	250,000											
3-27	360,000											
4-18	200,000			4.0	15.0	51.0	51.0	1.0	2.0	0.0	27.0	
4-26	260,000											
5-1	190,000			2.0	23.0	57.0	54.0	0.0	4.0	0.0	17.0	
5-8	170,000			6.0	35.0	32.0	32.0	1.0	2.0	0.0	24.0	
5-10	100,000	6.0										
5-16	110,000											
5-19	60,000											
5-21	51,000											Thiouracil discontinued
5-22	40,000	3.5		2.0	20.0	57.0	57.0	0.0	3.0	0.0	18.0	
5-24	51,000											
5-26	70,000	4.0		4.0	6.0	55.0	60.0	0.0	1.0	0.0	29.0	First convulsion
6-5	30,000	2.0		2.0	7.0	50.0	58.0	0.0	3.0	0.0	30.0	
6-7	42,000											
6-8	12,000	0.5	15.0	20.0	28.0	16.0	9.0	0.0	5.0	0.0	6.0	
6-9	13,500											
6-11	7,600	0.5	10.0	2.0	10.0	20.0	30.0	0.0	15.0	4.0	33.0	
6-12	7,400											
6-14	5,700											
6-15	6,200											
6-16	5,950											
6-18	5,750											
6-19	5,800											
7-3	1,900	Trace	18.0	0.0	0.0	0.0	0.0	0.0	33.0	0.0	49.0	24 hrs before death

a moderate myeloid immaturity with an increase in the basophils (8.0 per cent) and monocytes (18.0 per cent), and the bone marrow showed moderate myeloid hyperplasia. During the remission of the leukemic process Fowler's solution

(liquor potassii arsenitis) was begun and the dosage was gradually increased until the patient was taking 15 minims three times a day. The leukocyte count fluctuated between 11,200 and 60,000. Thiouracil 0.8 Gm daily was begun when the white cell count increased to 90,000 and the drug was continued for about 3 months. While the patient was receiving thiouracil the leukocyte count gradually increased to 300,000 and the spleen became palpable and gradually increased in size. The leukemic symptoms reappeared. The drug was then discontinued. Neither the blood nor bone marrow pattern was affected by thiouracil (table 2).

A man with chronic lymphatic leukemia (case 6) who was under observation for about one year was given thiouracil 0.9 Gm daily for about 75 days. No significant change was produced in the blood or bone marrow. Several months after discontinuing the drug he developed a deep and ulcerating lesion on the left posterior faucial pillar and complete bilateral deafness. The leukocyte count reached a peak of 260,000. Because of the generalized lymphadenopathy and splenomegaly several roentgen ray treatments were administered. Following this, the white cell count dropped to 60,000 and the lymph nodes and spleen decreased in size. The ulcerating oral lesion and deafness remained unchanged. The patient died in another hospital and no autopsy was obtained. There is no definite evidence to suggest that the thiouracil was the contributing factor in producing the ulcerating oral lesions or deafness in this case of lymphatic leukemia. The interval of several months from the time the thiouracil was discontinued speaks more for an exacerbation of the leukemic process.

In a fourth case of chronic myeloid leukemia (case 5) with a leukocyte count of 440,000 a daily dosage of 3.0 Gm was followed by an extreme leukopenia and unusual changes in the hemopoietic tissues. This case is reported in detail.

CASE REPORT (CASE 5)

H. W., a Negro housewife 50 years of age, was admitted to the hospital on February 2, 1945, with the complaints of prominence of the left upper abdomen, purple spots appearing on her extremities, loss of 25 pounds in weight and pains in her legs and arms. She had been well until March 1944, when she began to have aches in her thighs. About 3 weeks later elevated purple spots appeared on her legs, and continued to appear and leave. In the following summer she noticed abdominal fullness and enlargement, especially in the left upper quadrant. By the time of admission she had lost 25 pounds, although her appetite was good.

The past history was essentially negative.

Physical examination revealed a well-developed, well-nourished, Negro female not in any apparent distress. Ophthalmoscopic examination revealed a small hemorrhage with a white center in each eye. Anterior and posterior cervical, axillary, and inguinal lymph nodes were enlarged and palpable. The abdomen was rounded and prominent to the left, where the spleen could be palpated extending from the costal margin to the iliac crest, and from the left flank to the right of the umbilicus. It was firm and movable. The liver was not palpable. Raised purpuric areas, as much as 7 centimeters in diameter, were present on the thighs and right calf. The temperature was 97.6° F, the pulse 78, respirations 20. The weight was 125 pounds, the height 66 inches. The blood pressure was 110/76.

The urine had a specific gravity of 1.020 and contained a trace of albumin and a few epithelial cells. On admission the hemoglobin was 10 grams, red count 4,000,000 per cu. mm., and hematocrit 31 per cent. The leukocytes numbered 450,000 per cu. mm., with 1 per cent myeloblasts, 1 per cent promyelocytes, 39 per cent neutrophilic myelocytes, 32 per cent neutrophilic metamyelocytes, 2 per cent basophils, 2 per cent lymphocytes, 7 per cent eosinophils, 2 per cent stabs, 12 per cent polymorphonuclear neu-

trophils and 2 per cent monocytes. The Wassermann and Kahn tests were negative. Fasting blood glucose was 69 mg, nonprotein nitrogen 40 mg per 100 cc. Standard urea clearance was 34 cc per minute. Serum albumin was 3.8 per cent, serum globulin 1.9 per cent, and blood uric acid 2.9 mg per 100 cc. The basal metabolic rate was plus 44 per cent. An x ray of the chest showed elevation of both diaphragms, but normal heart and lungs.

Administration of thiouracil was begun on February 7, 1945, 1.0 gram three times daily.



FIG. 1. PERIPHERAL BLOOD SHOWING CHRONIC MYELOID PATTERN BEFORE THIOURACIL ADMINISTRATION

The temperature rose to 103.5° on February 14 and continued to be elevated. Thiouracil was stopped, but no effect on the fever was noted. A blood culture showed no growth. The drug was resumed 3 days after it had been withdrawn and the temperature gradually returned to normal on February 22.

The hemoglobin gradually dropped to 7 grams with a red blood count of 2,520,000. This anemia was treated with transfusions to the total amount of 4.5 liters of citrated blood. The hemoglobin at the time of discharge was 13.6 grams. Headache and muscular pains were treated with tablets of aspirin, phenacetin, and caffeine as needed, to the amount of 100 grains. Two grains of codeine were also administered. The basal metabolic rate was inconstant: plus 44 per cent, plus 33 per cent, plus 54 per cent, plus 41 per cent, and at the time of discharge plus 77 per cent. The nonprotein nitrogen rose to 55 and 62.7 mg per 100 cc and then declined to 56. The total white count fell to 180,000 by February 26 but rose to 360,000 by the end of March.

The patient was discharged to the outpatient department on April 14, 1945, and returned to the hospital on May 8, 1945. During the interval she continued the thiouracil as before 10 gram three times daily.

On final admission the patient was much weaker and had lost another 13 pounds in weight. The temperature was 99.2°, the pulse 80, respirations 18, weight 112 pounds. The blood pressure was 90/60. Otherwise no change was noted in the physical examination.

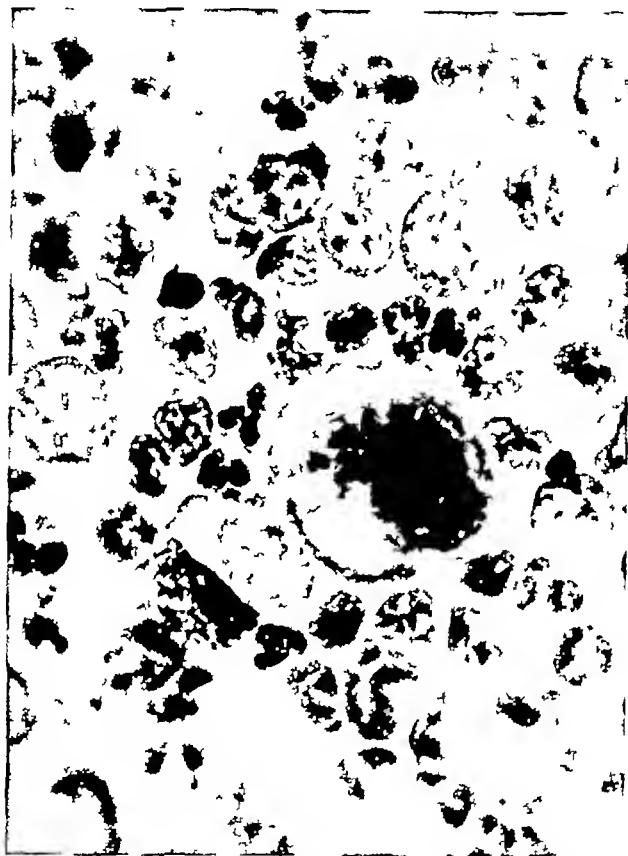


FIG. 2. BONE MARROW BEFORE THIOURACIL ADMINISTRATION. STERNAL PUNCTURE SHOWING MYELOID HYPERPLASIA AND MYELOID CELLS IN SEVERAL PHASES OF DEVELOPMENT.

Note the young megakaryocyte.

The hemoglobin was 13.0 grams, red cells 4,000,000, white cells 100,000. The uric acid was 3.6 mg per 100 cc, and rose terminally to 11.4 mg. The blood cholesterol was 224. The basal metabolic rate was plus 36 per cent. Thiouracil was continued at the same dosage as previously.

Epigastric fullness after meals was treated with tincture of belladonna with some apparent relief. For vomiting the patient was given one dose of cocaine $\frac{1}{4}$ grain orally preceded by $1\frac{1}{2}$ grains of seconal.

On May 18 the thyroid was biopsied. Thiouracil was stopped on May 25 after the patient had received a total of 274 grams. The microscopic examination of the biopsied tissue revealed an edematous stroma. The acini were oval or round in shape and varied markedly in size. The colloid stained unevenly and poorly. There was no evidence of hyperplasia or neoplasia.

The anemia required transfusions to the amount of 3 liters. The last blood transfusion was given on May 18. On May 19 the temperature rose to 102° and it rose to almost the same level on the succeeding 3 days. Fluoroscopy of the chest revealed some atelectasis of the right lower lobe. The fever subsided spontaneously. The patient had a convulsion on May 24, epileptiform in type, preceded by involuntary winking and tremor of eyelids. She was given $\frac{1}{2}$ grain of phenobarbital three times daily, but another convulsion occurred on May 25 and was treated with intravenous sodium amytal 5 grains. Although the winking occurred several times subsequently, convulsions were seemingly prevented by the sub-

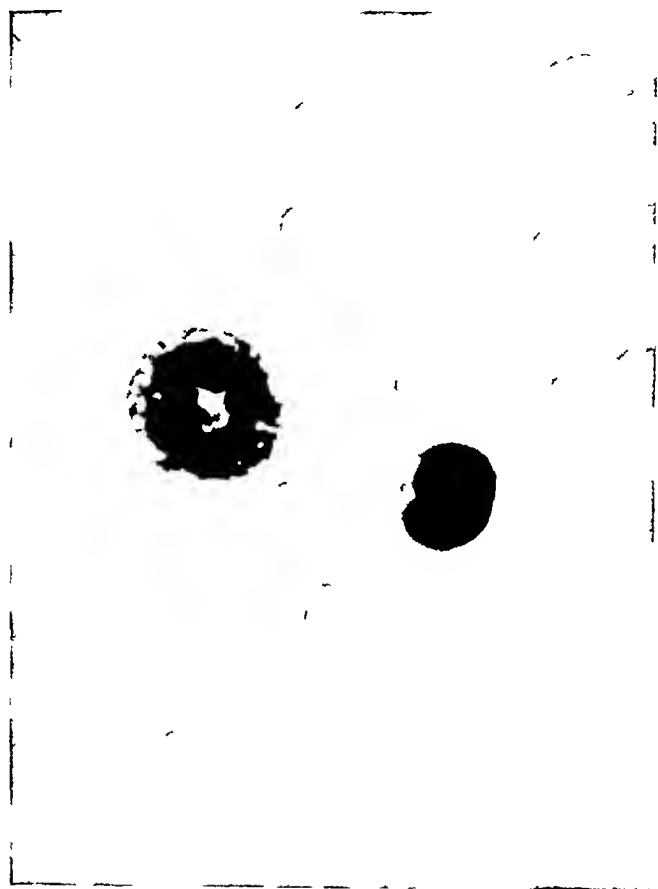


FIG 3. PERIPHERAL BLOOD FOLLOWING THIOURACIL THERAPY. TERMINAL BLOOD PICTURE SHOWING A NORMAL SMALL LYMPHOCYTE AND A BASOPHIL.

cutaneous administration of sodium phenobarbital 1 grain at such times, until June 11. On June 11 and again on June 12 the patient had convulsions both of which were treated with parenteral barbiturate. Because the twitchings became more diffuse and frequent, dilantin 0.1 gram three times daily, was started on June 14. An electroencephalogram taken on June 19 showed no focus of abnormal activity and no seizure discharges. There was 6-8 per second activity in all leads with rare bursts of 3-4 per second activity. Another convulsion occurred on June 25, and again on June 28 and 29. Three convulsions occurred on June 30, one on July 1, three on July 2. She became disoriented and irrational on July 3 and had one convulsion. During the early morning of July 5 she had three convulsions despite 3 grains of parenteral sodium phenobarbital.

At the time of readmission the white count was 110,000. On May 19 it had fallen to 60,000. The count of June 8 was 12,700. By July 3 it had fallen to the minimal level, 1,900.

The temperature began to rise on June 28 and reached a maximum of 104° (axillary) on July 5. At no time was there any oral or pharyngeal ulceration, even at the time of exitus.

The patient died in coma on July 5.

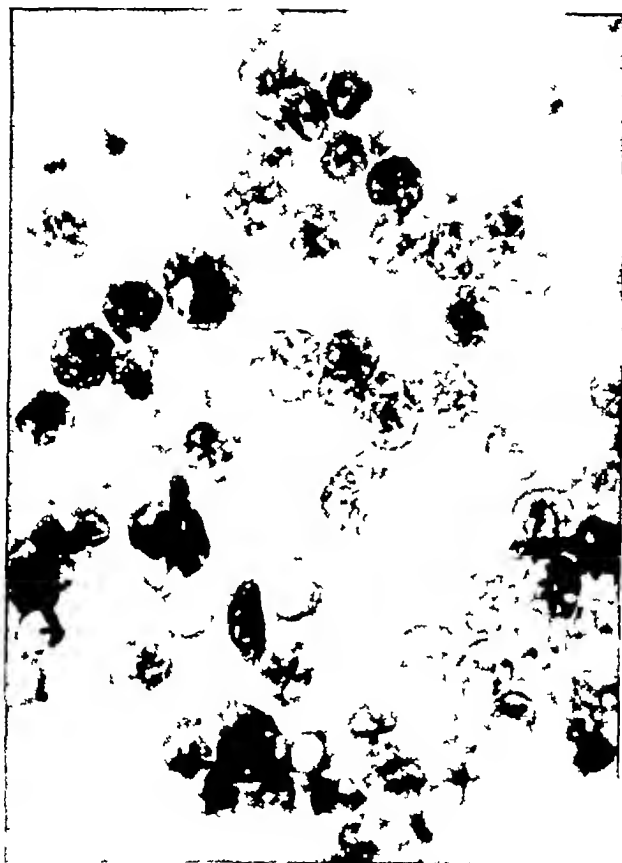


FIG. 4. BONE MARROW AFTER THIOURACIL AGRANULOCYTOSIS.
Note the hyperplasia of the myeloblastic tissue.

AUTOPSY

(Pertinent Findings)

The body was that of a well-developed, fairly well-nourished, Negro woman who had been dead about one half hour. There were two ecchymotic areas over the lateral aspect of the left arm.

Peritoneal Cavity The liver edge extended to approximately 3 fingerbreadths below the costal margin on the midclavicular line. The spleen filled the left upper quadrant, reaching the umbilical line. The mesenteric and retroperitoneal lymph nodes were moderately enlarged and rather soft. On section they showed yellowish-brown surfaces.

Pleural Cavities The lymph nodes in the mediastinum were slightly to moderately enlarged, having an appearance similar to those in the abdomen.

Heart The heart weighed 220 Gm and was somewhat softened. There was a large area of fibrous thickening of the pericardium over the anterior wall of the right ventricle. There were a few small areas of hemorrhage over the pericardial surface especially in the region of the right auricle. The subepicardial fat tissue was gelatinous and yellowish brown in color. The left ventricular wall measured 13 mm in thickness the right ventricular wall 2 mm.

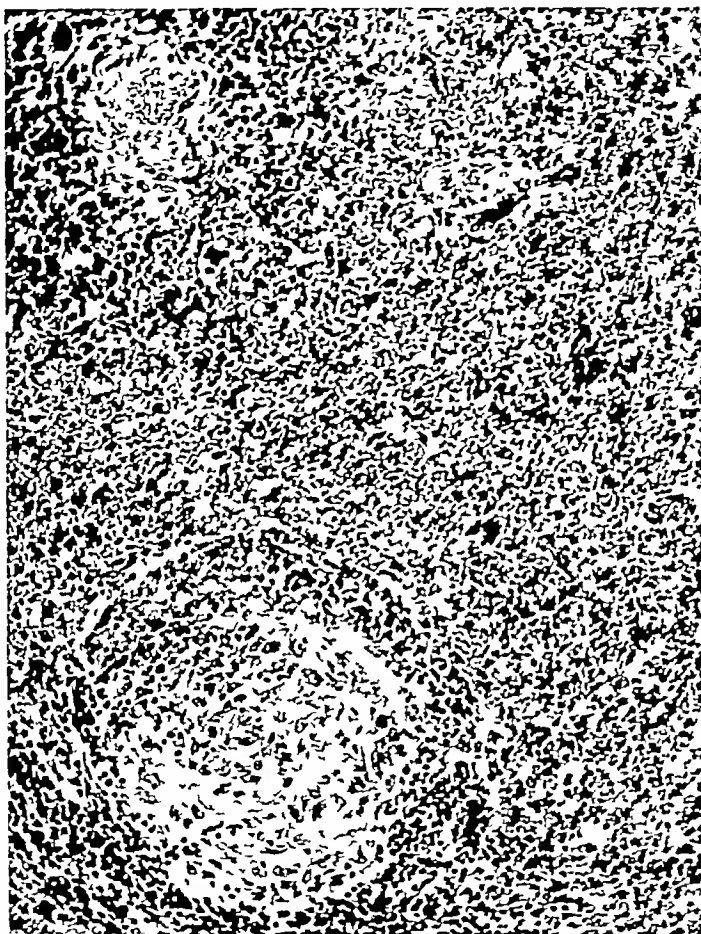


FIG. 5. SPLEEN (LOW POWER)

Note the marked congestion of the pulp and the paucity of blast cells. A follicle in the left lower corner presents a large center with degenerating reticulum cells.

Lungs There were occasional small areas of hemorrhage and atelectasis especially along the anterior edge.

Spleen The spleen was markedly enlarged and firm. It weighed 1250 Gm. The external surface was smooth, with a small area which contained fibrous tags. The cut surface presented a dark red color and was rather homogenous. The fibrous reticulum appeared slightly increased. Within the gastrosplenic ligament were a few nodular structures measuring up to 2 cm in diameter which presented on section a structure similar to that of the spleen.

Liver The liver was moderately enlarged weighing 2060 Gm. Numerous enlarged lymph nodes were

present at the hilus of the liver. On sectioning, these nodes presented yellowish brown surfaces with occasional small hemorrhagic areas.

Adrenals The adrenal glands showed no gross changes.

Kidneys The right kidney weighed 175 and the left 170 Gm. The capsules stripped with ease revealing a smooth, red tan surface. On section the cortical medullary markings were distinct and the cortex appeared somewhat swollen. In one of the pyramids of the left kidney was a small white nodule. The

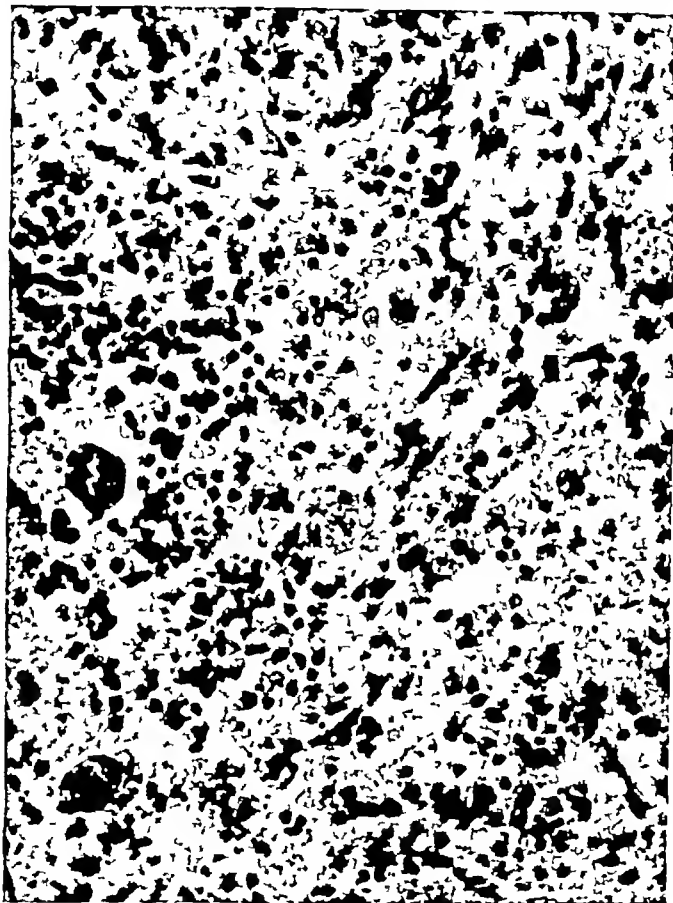


FIG 6 SPLEEN (HIGH POWER)

Abundant granular pigment is clearly visible either free or within macrophages. Practically no leukemic cells can be seen.

pelvic mucosa of both kidneys presented a few minute hemorrhages. Similar hemorrhages were present in the upper portions of both ureters. The remaining portions of both ureters and the urinary bladder appeared normal.

Thyroid Part of the right lobe was missing and what remained of thyroid tissue appeared fibrosed. The left lobe of the thyroid was rather small, measuring up to 4 cm. in its greatest dimension.

Head The skull was rather thick. The brain weighed 1300 Gm. and appeared normal on external

inspection. The dural sinuses were free of clots and the accessory sinuses and middle ears showed no gross changes.

Bone Marrow. The marrow of the sternum, ribs, and vertebrae in the lumbar region was rather pale in color and abundant.

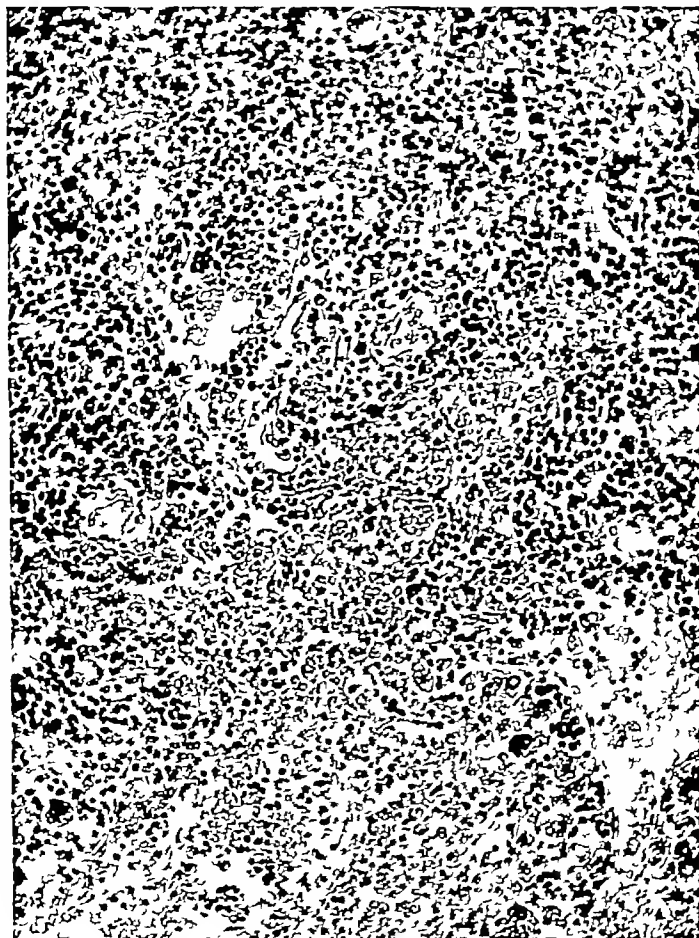


FIG 7 LYMPH NODE

There are numerous pigmented macrophages. The sinusoids are dilated and contain numerous leukemic cells.

MICROSCOPIC EXAMINATION

(Pertinent Findings)

Liver. The hepatic cell exhibited a moderate degree of cloudy swelling. Within the periportal spaces were small foci of lymphocytes, blast cells, and mononuclear cells with pale vesicular nuclei and distinct nucleoli (RE cells).

Spleen. The architecture was obscured. There were numerous hemorrhagic areas throughout the pulp, which was otherwise markedly congested and contained much granular brown pigment. There was an occasional follicle with degenerating, germinative cells (reticulum cells). The pulp contained small foci

of immature cells and an increased number of reticulo-endothelial cells. Section through an accessory spleen revealed a marked hyperemia. The sinusoids were dilated.

Lymph Nodes There was a diffuse hyperemia and a marked hyperplasia of the reticulo-endothelial cells. Numerous phagocytes with brown pigment were present. Occasional follicles were small and compressed, showing degeneration of the reticulum cells.

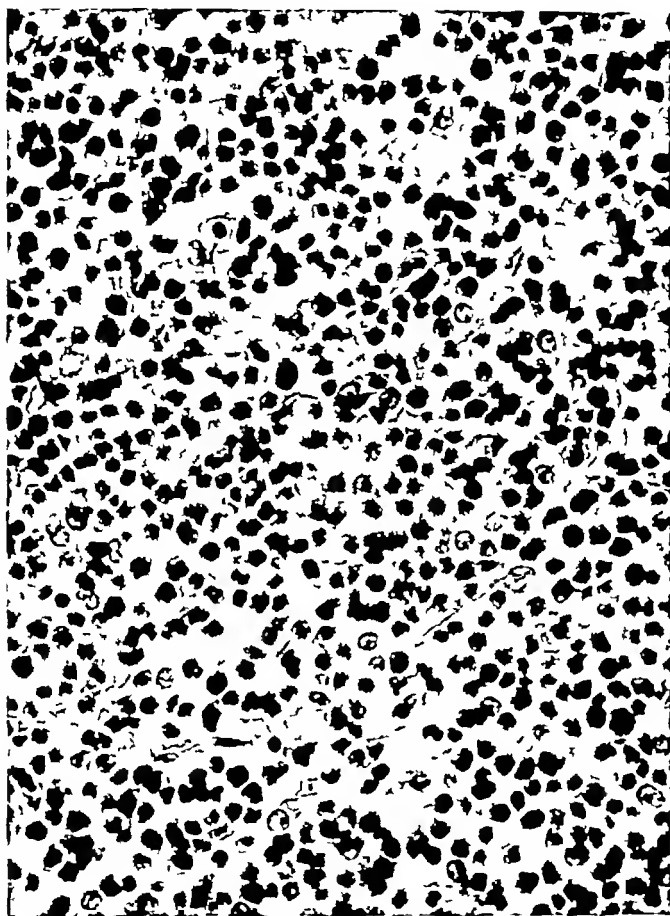


FIG. 8. BONE MARROW SHOWING MYELOID HYPERPLASIA OF MYELOBLASTIC TYPE.

Bone Marrow The cells were very numerous, rather homogenous in type, and of the blast type. Occasional megakaryocytes and several phagocytic (R. E.) cells were noted. Only occasional polymorphonuclear leukocytes were present.

Thyroid The alveoli were large and filled with colloid. In one area a marked foreign body reaction was noted around suture material.

Adrenals The cortex presented a few areas in the zona fasciculata where the cells were large, swollen, and presented a vacuolated cytoplasm.

Hypophysis There was a marked hyperemia and occasional foci of blast cells within the capsule.

Skin There were a few small focal hemorrhages within the derma.

Brain The gross and microscopic examination of the brain was conducted by Dr. Percival Bailey. Except for a few leukemic cells in the vessels, no abnormalities were observed.

The final anatomical diagnoses were (1) chronic myelogenous leukemia with involvement of the bone marrow, liver spleen (splenomegaly) kidneys, and lymph nodes (2) agranulocytosis (toxic), (3) multiple petechial hemorrhages of the serosal surfaces, (4) emaciation, (5) brown atrophy of the heart, (6) multiple small areas of foreign body reaction in the lungs, (7) emphysema of the lungs, moderate, (8) cloudy swelling of the myocardium, liver, and kidneys, (9) old surgical cervical incisions (partial thyroidectomy), (10) chronic perisalpingitis, right, (11) multiple accessory spleens, (12) small fibroma of the kidney

This case (H W) is of particular interest from two main aspects (1) the possible effect of thiouracil on the leukemic process, and (2) on the central nervous system The typical blood picture of chronic myelogenous leukemia appeared to be profoundly modified by thiouracil to the extent that an extreme neutropenic leukopenia resulted The terminal blood pattern in this case is not that usually seen in either a spontaneous remission or an acute exacerbation of a chronic myeloid leukemia, and it differs from that repeatedly observed in remissions induced by roentgen-ray treatment or liquor potassii arsenitis (Fowler's solution) The first effect of thiouracil in myeloid leukemia, as noted in this case, would appear to be a slowing of the rate of proliferation of the granulocytic elements, reflected in the peripheral blood by a gradual decrease in the total white blood count but with no change in the typical chronic myeloid blood pattern The second result of thiouracil might be one of actual granulocytic destruction The gradual disappearance of the granulocytes from the peripheral blood and the increase in the blood uric acid to 11.4 mg support the presence of two independent mechanisms in thiouracil leukopenia and neutropenia in leukemia, i.e. (1) an inhibition of granulopoiesis, and (2) a destruction of granulocytes At autopsy the spleen was found to be markedly enlarged (1250 Gm), but microscopically it presented a picture of marked hyperemia of the pulp with only small foci of blast cells, many showing atypical nuclei Cells of the granulocytic series were also strikingly absent from the bone marrow, lymph nodes, liver, and kidneys All these organs contained a moderate number of blast cells and presented a distinct hyperplasia of the reticulo-endothelial system The bone marrow, in particular, appeared markedly hyperplastic with the presence of megakaryocytes and a predominance of blast cells Erythropoiesis was decreased It is interesting to note that the final differential blood count consisted of 18 per cent myeloblasts, many atypical or toxic forms, 49 per cent lymphocytes, mostly small types, and 33 per cent basophils, both mature and immature forms There was a complete absence of neutrophils and eosinophils in the peripheral blood Apparently, the basophils were resistant to the toxic effect of thiouracil The lungs appeared grossly normal but microscopically presented a large number of small granulomatous lesions of the foreign body type These were explained on the possibility of aspiration of foreign material and probably were the cause of an episode of fever associated with pulmonary findings about a month before the death of the patient Finally, the thyroid failed to reveal any change attributable to thiouracil

The brain on external inspection and on cross section appeared entirely normal Microscopically, except for the presence of a few leukemic cells in the lumen of the blood vessels, no remarkable changes were noted From the electroencephalo-

gram made after the appearance of the convulsions, which showed no evidence of localized damage in accessible parts of the cortex and the lack of any morphologic change of note in the brain, it may be assumed that the clinical evidence of the convulsions resulted from the toxic effect of thiouracil on the central nervous system. In this connection the report of Haines and Keating⁴ is interesting. These investigators observed in 2 patients with severe recurrent exophthalmic goiter who were treated with thiouracil, toxic disturbances of the central nervous system consisting of myoclonic contractions of various muscles, and at the same time severe somnolence and confusion were present. Subsequent administration of the drug was followed by a resumption of the toxic disturbances of the central nervous system.

DISCUSSION

McGavack, Lombardi, and Schwimmer⁵ gave thiouracil to 78 patients with thyrotoxicosis and to 40 individuals without thyroid disease. No untoward response of any kind was observed in those individuals who did not have thyrotoxicosis. In those with thyrotoxicosis the toxic or unusual reactions were divided into two groups. (1) Incidental side effects such as rashes with pruritus, relative granulocytopenia, generalized edema or edema of eyes and ankles, diarrhea and dryness of the mouth and excessive thirst, these manifestations of a mild nature usually disappeared without altering the therapeutic regimen. (2) Manifestations of a severe nature which necessitated discontinuance of thiouracil and which included patients who developed severe febrile reactions with chills, generalized aching and widespread urticarial skin lesions, and agranulocytosis. These investigators reviewed the literature and found that of approximately 2,500 patients who had received thiouracil, 10 (0.40 per cent) had developed agranulocytosis and 4 of these (0.16 per cent) died. Fishberg and Vorzimer⁶ noted a definite and sudden granulopenia in 20 per cent of their patients and suggested the use of pyridoxine, vitamin B₆, in prophylactic doses of 150 mg daily by mouth, or 200 mg intravenously where severe drops in the leukocyte count have taken place.

From the hematologic point of view thiouracil produces two types of blood and bone marrow patterns. (1) Granulocytopenia in which there is a leukopenia, neutropenia, and a monocytosis. Here the monocytes appear to have their origin from the reticulum. The bone marrow is hyperplastic and shows a moderate to marked degree of granulopoietic immaturity. Immaturity is never carried to the stage of myeloblastic involvement. Ulcerating lesions of the oral cavity are not observed. Following temporary discontinuation of the drug, the blood completely recovers. In fact, there may be a temporary leukocytosis immediately following removal of the drug. (2) Agranulocytosis in which there is a severe leukopenia, complete absence of granulocytes including eosinophils and basophils. Here the bone marrow reveals an aplasia of the granulopoietic tissue. Erythropoiesis and megakaryopoiesis are affected very little. There are a few degenerated and atypical myeloblasts, a marked lymphocytic reaction and a relative increase in plasma cells and reticulo-endothelial elements. Pharyngeal ulcerations are frequently observed in thiouracil agranulocytosis. This type of patient usually dies in less than one

week from the first appearance of the ulcerations in the throat and tonsils. There is a complete aplasia of the granulopoietic elements in the bone marrow.

The clinical and hematological course followed by patients who develop thiouracil agranulocytosis is similar to that seen in agranulocytosis due to a number of drugs and chemicals.⁸ Plum⁹ and Rosenthal¹⁰ have described in detail the pathologic findings in agranulocytosis due to chemicals and many drugs, especially aminopyrine.

A comparison of the histological findings in cases of agranulocytosis described and illustrated by Rosenthal and the thiouracil agranulocytosis in our case of leukemia is interesting. He observed that in the lung, the alveoli were filled with red blood cells, bacteria, some large endothelial cells, and a few lymphocytes and fibrin. There was an absence of granulocytes. The lymph nodes revealed edema and marked hyperplasia of the reticulo-endothelial cells. The spleen, which may be enlarged and resemble the acute splenic tumor of infections, revealed on histological examination a definite hyperplasia of the reticulo-endothelial system. Plasma cells and lymphocytes were present, but no granulocytes. The bone marrow may be aplastic, normal, or hyperplastic. Rosenthal has also observed a reticulo-endothelial type of bone marrow. In the aplastic type there is an absence of granulocytes and myeloblasts with a marked increase in the number of lymphocytes which in some instances may take a follicular appearance, and a relative plasmocytosis. A number of investigators¹¹ have observed marrows in cases of agranulocytosis with numerous myeloblasts and a few myelocytes and rarely a few mature granulocytes. Degenerated myeloblasts have been described by some workers¹² in cases of recurrent agranulocytosis. In the reticulo-endothelial type of marrow a few myelocytes and myeloblasts and a marked hyperplasia of reticulo-endothelial elements are observed.

In comparison, in our case of leukemia that developed the extreme neutropenic leukopenia, the peribronchial tissue contained a few small accumulations of lymphocytes and epithelioid cells with giant cells. There was an absence of leukemic cell infiltration as commonly observed in cases of leukemia. The architecture of the spleen was obscured by numerous hemorrhagic areas throughout the pulp, which was otherwise markedly congested and contained much granular brown pigment. There was an occasional follicle with degenerating germinative cells (reticulum cells), and the pulp contained small foci of immature cells with an increased number of reticulo-endothelial elements. In the lymph nodes a diffuse hyperemia and a marked hyperplasia of the reticulo-endothelial cells were observed. Some follicles were small and compressed and showed degeneration of the reticulum cells. The bone marrow was hyperplastic and consisted mostly of myeloblasts, many atypical, and a number of reticulo-endothelial elements. Granulocytic elements were practically absent. Erythropoiesis was depressed and a few megakaryocytes were present.

An interesting observation concerns the persistent splenomegaly after the organ had been practically depleted of leukemic cells as noted at autopsy. It is generally agreed that splenomegaly in leukemic states is due for the most part to proliferation and infiltration of leukemic elements. Apparently, hyperemia and hyperplasia

of the reticulo-endothelial elements are major factors in producing an enlarged spleen in chronic myeloid leukemia. This also explains the cause of the enlarged liver in the presence of a minimal leukemic infiltration of the organ.

Astwood¹³ first employed thiourea and thiouracil in the treatment of patients with toxic goiter and found that the drug produced complete remission of symptoms and return of the basal metabolic rate to normal in most patients. These antithyroid drugs inhibit the function of the thyroid gland and, as Williams¹⁴ has pointed out, this is true in spite of the fact that they may increase the work of the gland as evidenced by the marked hyperplasia and hypertrophy which they produce in the thyroid. In patients with leukemia treated with thiouracil there is no clinical improvement, the basal metabolic rate remains elevated, and there is no morphologic thyroid hyperplasia.

The most dangerous extrathyroid effect of thiouracil therapy is agranulocytosis. The findings in the blood and bone marrow have been described. The pathophysiological mechanism involved in agranulocytosis is not entirely clear. Plum⁹ studied the blood and bone marrow simultaneously in recovered cases of agranulocytosis following the administration of aminopyrine. He observed that the myeloblasts, promyelocytes, myelocytes, and metamyelocytes gradually diminished after a few days and at the same time there was a relative increase in mature granulocytes. With the severity of the process the more mature granulocytes gradually became completely depleted, and finally in the very severe and fatal cases the marrow was hypocellular. There was no indication of a maturation arrest,¹¹ but rather, a hypoplasia of immature granulocytes. According to this mechanism of agranulocytosis, the toxic factor affects, first, the granulocytic precursors followed by the mature granulocytes and, finally, a depletion of all granulocytes with no cells being formed. This is reflected by an agranulocytosis in the peripheral blood. Gargill and Lesses¹⁵ also support this thesis and suggest the destruction of leukocytes in the blood stream as a possible mechanism of the leukopenia as discussed by Lawrence.¹⁶ Experimentally, Warren¹⁷ was able to show that thiouracil in 100 mg per cent concentration induced a small but significant inhibition of respiration of rabbit bone marrow cells, the effect upon the myeloid elements being more striking. The myeloid elements (mostly myelocytes) were found to be more sensitive to the action of the drug than the more mature polymorphonuclear cells found in the peritoneal exudates of rabbits. Attempts to protect the marrow from the depressant action of thiouracil on respiration by adding pyridoxine or dilute liver extract, all yield negative results. This is of interest since these products have been suggested for the prevention of granulocytopenia induced by thiourea and its derivatives.¹⁸

Williams and his associates¹⁹ have reported detailed studies on the absorption, distribution, and excretion of thiouracil in experiments on rats and man. They found that thiouracil is rapidly absorbed from the gastrointestinal tract and is readily excreted in the urine. With dosages ranging from 0.2 to 1.2 Gm daily, the concentration of the drug in the blood varied from 0.8 to 6.4 mg per 100 cubic centimeters, while the daily excretion in the urine varied from 16 to 618 mg. The distribution of thiouracil in different elements of the blood was studied in

4 individuals who had been treated with the substance for several days. In 3 subjects, the blood cells were found to contain about seven times as much as the plasma, in 1 subject the cells contained twice as much as did the plasma. Although the red cells were found to possess two or more times the amount present in the white cells, the average amount of thiouracil per cell was much greater in the white cells than in the red cells. These investigators carried on some interesting experiments on the absorption of thiouracil by blood cells *in vitro*. Among the cases studied were the leukocytes from the blood of 3 cases of leukemia. Fifty cc of blood were obtained from each case of acute myeloid leukemia, chronic myeloid leukemia, and chronic lymphatic leukemia. To the blood were added 10 drops of a 20 per cent solution of potassium oxalate and enough of a 20 mg per cent solution of thiouracil to make a final concentration of about 4 mg per cent. The mixture was incubated at 38°C for 1 hour and immediately thereafter the estimations of thiouracil were begun. They found that the higher the white count the larger the amount of the drug present in the white cells, whether the cells were almost entirely lymphocytes or whether they were granulocytes. Although the average quantity of thiouracil removed by individual granulocytes was greater than that removed by lymphocytes, the latter cells absorbed more of the drug in comparison to their size. The leukocytes of the acute leukemic patient removed the same amount per volume of cells as did the chronic myelogenous leukemia cells, but the amount per cells was less in the former group. The erythrocytes of the leukemic patients did not remove as much thiouracil per volume of cells as did the red cells of the nonleukemic patient. Finally, it was found that the final concentration of the drug in the plasma was less in the chronic leukemic blood than in the nonleukemic blood. These findings indicate that the leukemic cells of both myeloid and lymphatic leukemia are more active in ingesting thiouracil from the plasma than are nonleukemic leukocytes. Further, the erythrocytes are less active than the white cells in the ingestion of thiouracil. A case of chronic lymphatic leukemia who received thiouracil for several days preceding death was found at necropsy to have some of the substance in essentially all the tissues of the body with very large quantities in the bone marrow, greater than that in any other tissue.

It is interesting to note (case 5) that there was an interval of 6 weeks between the onset of extreme leukopenia and the stopping of thiouracil. The gradual and persistent drop in the white blood count and disappearance of granulocytes from the peripheral blood is difficult to dissociate from a cumulative effect and retention of some thiouracil by the large quantity of leukemic tissue. Experimentally, Williams, Kay, and Jandorf¹⁹ have shown that within certain limits the more tissue present, the less the total destruction of thiouracil. This may in part explain the final production of leukopenia and neutropenia after thiouracil was discontinued.

From the blood and autopsy studies in the case reported above there is evidence that the leukemic process was partially altered although the clinical state was not beneficially affected. There was no evidence to indicate that the thiouracil had produced an exacerbation of the process as one frequently observes in cases of acute exacerbation of chronic myeloid leukemia. The terminal blood pattern was that of a chronic myeloid leukemia from which all the neutrophils and eosinophils

had been removed, leaving a relative increase in myeloblasts, basophils, and lymphocytes

The basophils (mast cells) were apparently unaffected by thiouracil, and in fact became increased during thiouracil administration. A similar type of basophilia is observed in cases of chronic myeloid leukemia in which a remission had been induced with roentgen therapy and liquor potassii arsenitis (Fowler's solution). The origin and the distribution of the mast cells (basophils) could not be ascertained in this material. It is well known that the basophil granulation is soluble in water and that an adequate evaluation of the morphology of the human mast leukocyte can only be obtained in properly fixed and stained preparations, preferably in films fixed in 100 per cent alcohol and stained in 50 to 80 per cent alcohol thionine stain.²⁰

In the past, many agents have been tried in an attempt to influence human leukemia. With the exception of the remissions induced by liquor potassii arsenitis, roentgen therapy, and radioactive substances, all such attempts have uniformly met with failure. My associates and I (L. R. L.) have tried a number of other medical and surgical procedures in cases of leukemia in an attempt to alter the leukemic process. These included aminopyrine,²¹ colchicine,²² and total thyroidectomy,²³ the latter in order to produce myxedema and influence the metabolism of hemopoiesis in leukemic conditions. No clinical or hematological improvement of lasting value was noted. Thiouracil may occasionally alter the leukemic process and in so doing produce the other extreme, a neutropenic leukopenia, an effect that is not clinically beneficial. It can be assumed that idiosyncrasy or hypersensitivity does not play a part in thiouracil leukopenia and neutropenia in leukemia.

CONCLUSIONS

1. Thiouracil, which is occasionally productive of agranulocytosis, is of no value in the treatment of leukemia, even when given in very large doses.
2. In one case of chronic myeloid leukemia given 274 Gm. of thiouracil for 90 days, an extreme leukopenia developed 6 weeks after the drug was discontinued. The patient developed toxic disturbances of the central nervous system. Both of these effects may have been related to the thiouracil administration.
3. In cases of chronic myeloid leukemia with a high percentage of basophils in the peripheral blood, the basophils appear to be resistant to the toxic effect of thiouracil and in this respect simulate the blood pattern observed in remissions induced by roentgen therapy.
4. Thiouracil in sufficient doses may inhibit granulopoiesis and destroy granulocytes.

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EDITORIAL

CHEMOTHERAPY OF LEUKEMIA AND RELATED DISEASES

OF THE various disorders of the blood and blood-forming organs, the leukocytic neoplasms and leukemia remain by all odds the chief problems. Brilliant studies have been made in the delineation and the therapy of the deficiency syndromes, and slow but steady progress continues in the control of such disorders as polycythemia, the hemolytic syndromes, the hemorrhagic diseases, and in the disorders in which a splenic dysfunction is preeminent. In leukemia quite the reverse is unfortunately true. This is particularly so in the acute cases, in which, once the diagnosis has been definitely made, the patient's death-warrant is signed.

In all probability, leukemia is a rapid, irreversible, proliferative process which affects one of the white cell forming tissues. The chronic forms respond rather well and for a period of one to several years following treatment with x-rays or radioactive phosphorus. Benzol, which was one of the first therapeutic agents to be used, was discarded perhaps prematurely. A number of good reports of several years ago stress its therapeutic value, which is said to compare favorably with that of x-ray. The report of Flory, Steinhardt, and Furth in this issue may stimulate further study of this simple chemical in treatment. The group at the Jefferson Medical School has succeeded in extracting materials from the urine and the organs of leukemic patients which they are utilizing for treatment of acute cases. Lymphocytic urine extract is being used in myeloid cases and vice versa. The results thus far have been of sufficient interest to warrant further work. The widely touted A C S (anti-reticular cytotoxic serum) of the Russians has failed to affect either leukemic or lymphosarcomatous processes.

Mustard gas, which was ready for instant use during the recent war, was studied for its possible hematologic effects. These studies, a preliminary report of which by Gilman and Phillips¹ appeared recently, revealed that the sulfur and nitrogen mustards were productive of a type of action on cells which can be likened to that of no other chemical agent but which resembles in many ways that of x-rays. An official statement by Rhoads² dealing with clinical aspects has been formulated.

The cellular susceptibility appeared to be directly related to the degree of proliferative activity, thus the cells of the blood-forming organs were found to be greatly affected with a resultant pancytopenia and lymphocytopenia. The primary mechanism of these chemicals was the inactivation of essential cellular enzymes, notably the phosphokinases. The mitotic activity of rapidly proliferating cells was profoundly affected by minimal or threshold doses, and this effect might persist for several days in successive crops of proliferating cells. Gilman and Phillips state that no other class of chemical agents has been shown to have such specificity of action on chromosomal mechanisms. In threshold doses they act directly on the intimate structure of chromosomes.

Nitrogen mustards in the forms of hydrochloride salts were readily dissolved in water or sterile normal salt solution for intravenous administration and were first

used clinically by Alfred Gilman, Louis S. Goodman, G. E. Lindskog, and John Dougherty at Yale Medical School in a group of six patients with terminal neoplastic disease. The results were sufficiently impressive to warrant trial by several groups of investigators in various parts of the country.

Although the results in the various clinics have varied to some extent, there can be no question but that the intravenous injection of very small doses (5-10 mg) of either the *bis* or *tris* form of the nitrogen mustard often causes profound changes in leukemia, lymphosarcoma, and particularly in Hodgkin's disease. The drug must be given with extreme caution, since it not only affects leukocytic tissues, but causes a varying degree of anemia and thrombocytopenia. Other toxic effects are vomiting and venous thrombosis. In my own experience the use of these chemicals has been disappointing in the leukemias, but in cases of Hodgkin's disease, some of which had become completely refractory to x-ray therapy, remarkable remissions not infrequently took place. The drug certainly requires further clinical trial under carefully controlled conditions. Gilman and Phillips conclude their article with the following cogent statement: "It may be hoped that the previous successes which have characterized the evolution of chemotherapeutic agents by chemical alterations of a parent compound may be duplicated in the case of the β -chloroethyl amines. The result would be a compound having a sufficiently specific toxic action for certain types of proliferative cells to possess therapeutic value."

WILLIAM DAMESHEK, M.D.

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ABSTRACTS

ABNORMAL BLOOD PIGMENTS

CLEMENT A. FINCH, M.D., AND JOSEPH F. ROSS, M.D.

THE BLOOD PIGMENTS, THE PROPERTIES AND QUANTITATIVE DETERMINATION WITH SPECIAL REFERENCE TO THE SPECTROPHOTOMETRIC METHODS *H. O. Michel and J. S. Harris* J Lab & Clin Med 25 445-463, 1940

The authors present an orientational discussion of the nature and chemical properties of the various blood pigments derived from hemoglobin. The absorption spectra of these substances and their reactions to certain chemicals are described. Particularly useful is their outline of the qualitative identification by simple spectroscopy of methemoglobin, sulfhemoglobin, hematin, and pseudomethemoglobin (methemalbumin). Quantitative methods of analysis i.e., gasometric, combined gasometric and colorimetric and spectrophotometric are reviewed. The authors present a spectrophotometric method with experimental data based on extinction coefficients for the determination of mixtures of sulfhemoglobin, methemoglobin and oxyhemoglobin.

C A F

A RAPID SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF METHEMOGLOBIN AND CARBONYL HEMOGLOBIN IN BLOOD *B. L. Herecker and F. S. Brackett* J Biol Chem, 152 669-677, 1944

This method utilizes the same principle as the method employed by Evelyn and Malloy (J Biol Chem 126 655, 1938). The instruments used were the Coleman spectrophotometer and especially constructed photometers. Measurements were made at 8000 Å and at 4965 Å. Results by the method were compared to the Van Slyke gasometric determinations. The procedure is simple, and subject to an error of about 1%. This method appears to be the most satisfactory for routine use.

C A F

THE EFFECT OF METHEMOGLOBIN ON THE EQUILIBRIUM BETWEEN OXYGEN AND HEMOGLOBIN *R. C. Darling and F. J. W. Roughton* Am J Physiol, 137 56-68, 1942

Experiments were performed on ox and on human blood. The methemoglobin was made by aerobic oxidation by ferricyanide or by nitrite. The presence of methemoglobin caused a shift in the oxygen dissociation curve to the left, qualitatively the same but not as great as that produced by carbon monoxide. The shift was reversible once the methemoglobin had reverted to the ferrous form.

The authors conclude that this newly discovered effect means that in methemoglobinemia the tissues are liable to anoxemia not only from loss of oxygen capacity of the blood, but also from increasing difficulty in the unloading from the blood of such oxygen as is available.

The mechanism is believed to be the formation of compounds intermediate between reduced hemoglobin (entirely ferrous) and methemoglobin (entirely ferric), the conversion of one or more of the four ferrous atoms in the hemoglobin molecule to the ferric valence leading to an increased affinity of the remaining ferrous atoms for oxygen.

C A F

THE COMPARATIVE ANOXEMIC EFFECTS FROM CARBON MONOXIDE HEMOGLOBIN AND METHEMOGLOBIN *D. Lester and L. Granberg* J Pharm & Exper Therap 81 182-188, 1944

Carbon monoxide hemoglobin and methemoglobin, both functionally inert would seem to differ physiologically only in the degree to which they shift the oxygen dissociation curve and interfere with normal tissue oxygen exchange. In this study the levels of these pigments at which death occurred are re-

ported. Cats became unconscious and died when 66 to 71% of hemoglobin had been converted to carbon monoxide hemoglobin. Cats and dogs could tolerate more than 80% conversion of hemoglobin to methemoglobin without becoming unconscious. The asphyxial effects of methemoglobin thus were significantly less than those of carbon monoxide.

C A F

CONGENITAL IDIOPATHIC METHHEMOGLOBINEMIA. FAVORABLE RESPONSE TO ASCORBIC ACID THERAPY. R F Steers and J B Ryon. Arch Int Med, 76: 299-307, 1945

The article includes the most complete tabulation of clinical reports of this rare disease, summarizing 18 cases and adding one. (In the opinion of the reviewer the case reports of Miller, and of Schwartz and Rector should be excluded, their discrepancies are mentioned by the authors.)

In the majority of cases the cyanosis had been first attributed to a congenital cardiac defect. Symptoms were mild, including dyspnea on exertion, tachycardia, and headache. Methemoglobin levels varied from 10 to 57% of the total hemoglobin. The mean was 30 to 40%.

The authors reported one case and demonstrated the efficiency of ascorbic acid and methylene blue in reducing the degree of methemoglobinemia. Before ascorbic acid therapy the blood of their patient had no capacity to revert methemoglobin on standing. Normal plasma was able to effect some slight reduction of the methemoglobin of the patient's cells. After four days of treatment there was 1.6 Gm. reduction of methemoglobin in the patient's blood on standing 24 hours. Equal disappearance of methemoglobin at this time was found with the red cells suspended in either plasma or sodium chloride, which pointed to the operation of an intracellular rather than a plasma reducing substance. They felt that adding ascorbic acid partially replaced the defective cell reducing system.

C A F

FAMILIAL IDIOPATHIC METHHEMOGLOBINEMIA AND ITS TREATMENT WITH ASCORBIC ACID. H Bartlett, Q H Gibson, D C Harrison and J McMurray. Clin Sci, 5: 145-157, 1945

The effect of ascorbic acid therapy, 100-200 mgms. per day, in one case of familial idiopathic methemoglobinemia was described in detail. The initial level of 7.3 grams methemoglobin fell to 0.8 grams over a period of 30 days while the total hemoglobin level remained fairly constant. The decrease in ferric pigment paralleled the rise of blood ascorbic acid which was initially low. The same reducing action of ascorbic acid was demonstrated *in vitro* with hemolyzed or non-hemolyzed blood aerobically and anaerobically.

The authors felt that the increase in ascorbic acid concentration coincided with a decrease in methemoglobin concentration, was consistent with Gibson's report that the reaction may be expressed by a bimolecular formula (that the rate of reduction of methemoglobin is proportional to the product of the concentration of the two reactants).

The normal erythrocyte is able to reduce methemoglobin to hemoglobin in the presence of either lactate or glucose. Methylene blue greatly catalyzes this reaction. The patient's blood shows a greatly decreased ability to effect this reversion with either substance. Methylene blue in the presence of glucose produced a normal catalytic reduction in the patient's red cell but did not catalyze with lactate as a substrate. This was interpreted as a defect in the enzyme system of the red cell which functions to convert methemoglobin to hemoglobin.

C A F

CYANOSIS IN INFANTS CAUSED BY NITRATES IN WELL WATER. H H Comly, J A M A, 129: 112-116, 1945

Two infants are reported who on a formula containing holed water developed severe cyanosis which was determined spectrophotometrically to be due to methemoglobinemia. The administration of 100 cc. case 1: 1 mgm./kilo. and in the second of 1.5 mgm./kilo. of methylene blue within a few minutes reverted the abnormal pigment to hivalent hemoglobin. The well water used in both cases contained large amounts of nitrates and was heavily polluted with bacteria, presumably nitrate formers. The ingested nitrate was apparently converted by bacteria of the intestine to nitrite which, following absorption into the blood stream, produced methemoglobinemia.

C A F

DYE POISONING IN THE NURSERY A REVIEW OF 17 CASES *J Granbarth, C J Bloom, F C Coleman and H N Solomon J A M A 128 1155-1157, 1945*

Another episode of aniline poisoning through skin absorption is described Seventeen babies wearing new aniline stamped diapers developed cyanosis, fifteen other babies with previously washed diapers did not Many of the affected infants apparently developed anemia Three died of infection and one of intracranial hemorrhage Methemoglobinemia was assumed to be present and treatment consisted of methylene blue, oxygen and 5% carbon dioxide, and transfusions The authors felt that methylene blue was without apparent effect and expressed skepticism as to its value in the treatment of methemoglobinemia This opinion seems unwarranted in the absence of spectroscopic data identifying the pigment as methemoglobin

C A F

METHÄMOGLOBIN, INNENKÖRPER DER ERYTHROCYTEN UND ANÄMIE *W Heubner Klin Wchnschr, 20 137-141, 1941*

In a review article Heubner discusses the concept that the formation of methemoglobin is a reversible oxidation process without associated hemoglobin breakdown An entirely different type referred to as deep oxidation is represented by the splitting open of the porphyrin ring and the production of verdohemochromogen Heubner believes sulfhemoglobin is a product of deep oxidation and that it does not contain sulfur (His ideas on sulfhemoglobin seem poorly substantiated and are at variance with most present opinions)

Considerable attention is given to the early descriptions by Heinz, Ehlich and others of the inner bodies in erythrocytes, and of their production by hemolytic aromatic compounds Inner bodies and anemia may be produced with little or no methemoglobinemia methemoglobin is not a stage in the development of the inner body and anemia

On the basis of rather inadequate *in vitro* evidence the author feels that hemolytic agents may inactivate erythrocyte catalase and thus leave the cell vulnerable to oxidation by hydrogen peroxide

While many assertions of the author cannot be accepted, his general thesis of separating methemoglobinemia from hemolytic anemia is undoubtedly valid and well documented

C A F

THE CONTROL OF METHEMOGLOBINEMIA WITH METHYLENE BLUE *W B Wendell J Clin Investigation, 18 179-185 1939*

The normal rate of reconversion of methemoglobin (produced by injection of sodium nitrite) to hemoglobin by the red cell was 0.028 vol %/min Methylene blue administered intravenously at the peak of methemoglobin formation increased the reversion rate about five fold

Methemoglobin was demonstrated in the blood of patients on sulfonamide Intravenous injection of 0.1 cc to 0.2 cc per kilo of body weight of a 1% aqueous solution of methylene blue converted in 45 minutes all of the methemoglobin into functionally active hemoglobin The duration of this action was 12 to 24 hours and no toxic effects were observed

C A F

STUDIES ON SULFHAEMOGLOBIN *R Lemberg, H F Holden, J W Legge, and W H Lockwood Aust J Exper Biol & Med Sci 20 161-167, 1942*

This study was directed at the confused subject of the structural nature of sulfhemoglobin The authors uphold the idea that the compound is composed of a closed porphyrin ring They differentiate spectroscopically the open ring reduced choleglobin from sulfhemoglobin by its behavior to alkali or carbon monoxide Sulfhemoglobin was reconvertible into protohemochromogen Artificially produced sulfhemoglobin generally contained as a by product 20 to 50% of choleglobin The properties of this decomposition product have led to confusion In clinical sulfhemoglobinemia little or no choleglobin was found, although it was present in the blood of rats fed sulfur and acetanilide

C A F

METHEMOGLOBIN A NORMAL CONSTITUENT OF BLOOD *W D Paul and C R Kemp Proc Soc Exp Biol and Med, 56 55-56 1944*

By a spectrophotometric method methemoglobin was found in 99 of 100 hospital patients in amounts

ranging from 0.01 to 0.5 Gm /100 cc blood. In 20 blood donors the range was from 0.03 to 0.13 Gm %. This confirms the previous observation of Ammundsen, E (J Biol Chem, 138: 563-570, 1941) and has been corroborated by F. J. W. Roughton, R. C. Darling and W. S. Root (Am J Med Sc, 208: 132, 1944) and by D. L. Drabkin and C. F. Schmidt (J Biol Chem, 157: 69-83, 1945) using gasometric methods.

The demonstration of methemoglobin in the blood of normal individuals helps to establish the concept of an equilibrium between the reduced and oxidized forms of hemoglobin. The extreme degree that the hemoglobin-methemoglobin equilibrium has been displaced in favor of the reduced form is an expression of the efficiency of the intracellular reducing system of the mammalian red cell.

C A F

METHEMALBUMIN. PART I. CLINICAL ASPECTS. N. H. Fairley, M.D. Quart J Med, 10: 95-114, 1941

The blood of patients with severe hemolytic anemia (e.g., blackwater fever, and paroxysmal nocturnal hemoglobinuria) is of peculiar chocolate color and the plasma is brown. This discoloration is caused by the presence of an abnormal pigment, methemalbumin, in the plasma. Fairley and Bromfield identified this pigment and differentiated it from methemoglobin and sulfhemoglobin, which in contrast to methemalbumin, are practically always intra-erythrocytic pigments. Methemalbumin is found in the plasma when intravascular hemolysis has been excessive. It is apparently formed in the plasma by the combination of the heme complex (derived from the destruction of hemoglobin) with plasma albumin. Cases described as hematinæmia are actually cases of methemalbuminæmia, since hematin in plasma immediately combines with albumin to form methemalbumin. It can readily be differentiated from other heme pigments by its characteristic alpha absorption maximum at 623-624 μ (methemoglobin 630 μ , sulfhemoglobin 618 μ), and by the effect of certain chemical agents on its alpha absorption bands.

Fairley believes that all cases of hemolytic anemia may be classified into three groups accordingly as they show a) hyperbilirubinemia alone, b) hyperbilirubinemia and methemalbuminæmia, c) hyperbilirubinemia, methemalbuminæmia, and haemoglobinemia. He suggests that hyperbilirubinemia in the absence of methemalbuminæmia suggests intracellular (reticulo-endothelial) blood destruction, whereas methemalbuminæmia implies lysis of corpuscles in the circulating blood.

J F R

METHEMALBUMIN. PART II. ITS SYNTHESIS, CHEMICAL BEHAVIOUR, AND EXPERIMENTAL PRODUCTION IN MAN AND MONKEYS. N. H. Fairley, M.D. Quart J Med, 10: 115-138, 1941

Methemalbumin was invariably formed *in vitro* when purified alkaline hematin (ferric) was added to plasma or to the albumin fraction of plasma or to crystalline albumin. It was not formed when hematin was added to euglobin, pseudoglobulin, seroglycoid or globuglycoid. The methemalbumin so formed was identical spectroscopically and chemically with that found in the plasma of patients with hemolytic anemia. Numerous chemical and physical studies indicated that methemalbumin is a definite chemical compound consisting of a prosthetic group, oxidized hematin (ferric), and a protein component, native serum albumin. On reduction, hemalbumin, a ferrous compound, was formed, but this compound differed from hemoglobin in being unable to combine loosely with oxygen.

Injection of alkaline hematin (ferric) or of reduced alkaline hematin (ferrous) into human subjects and into monkeys resulted in the immediate formation of methemalbumin. Interestingly enough, Fairley reports that there was no rise in plasma bilirubin following the injection of hematin, observations in conflict with those of Pass, Schwartz and Watson.

Fairley suggests that the mode of extracorporeal hemoglobin disintegration following intravascular hemolysis in man may occur as follows: hemoglobin is split in the circulation into globin and reduced hematin (ferrous), the latter is immediately oxidized to haematin (ferric) which combines with serum albumin to form methemalbumin. The subsequent fate of the methemalbumin is not discussed.

J F R

METHEMALBUMINÆMIA DURING COMBINED THERAPY WITH PAMAQUINE AND QUININE. W. D. Blake, C. G. Zulrod and M. Rosenfeld. Fed Proc, 5: 167, 1946

Methemalbumin appeared consistently in the serum of patients receiving pamaquine and quinine in combination for the treatment of malaria, although this pigment was not produced in patients receiving either of the two drugs alone in the same dosage. The concentration of methemalbumin progressively increased during fourteen days of combined therapy.

There were concurrent disturbances in pigment metabolism consisting of methemoglobin production in the erythrocytes increased fecal urobilinogen and increased fecal and urinary coproporphyrin

J F R

THE CONVERSION OF HEMATIN TO BILIRUBIN FOLLOWING INTRAVENOUS ADMINISTRATION IN HUMAN SUBJECTS *I J Pass, S Schwartz and C J Watson* J Clin Investigation 44 283-291 1945

The normal conversion of hemoglobin to bilirubin is supposed to occur with an opening of one of the methene linkages of the porphyrin ring and the formation of an intermediary pseudohemoglobin a biliverdin iron globin compound Hematin, a compound in which the porphyrin ring is still intact but which has been split free from the globin fraction of hemoglobin is not formed in the usual *in vivo* mechanism of hemoglobin degradation Fairley showed however that hematin is formed when excessive intravascular hemolysis occurs and that it immediately combines with plasma albumin to form methemalbumin

The way in which the body rids itself of hematin and methemalbumin is obscure but of some fundamental interest because of the frequency of its formation in severe hemolytic states and because malarial pigment is identical with hematin Duesberg Bingold Fairley and other investigators have reported that once hematin and methemalbumin were formed intravascularly they were not converted to bilirubin Pass Schwartz and Watson present definite evidence that hematin is converted into bilirubin Following injections of hematin into human subjects an increase of fecal urobilinogen occurred which was proportional to the amount of hematin given The serum bilirubin was not uniformly elevated however, and methemalbumin persisted in the serum for prolonged periods of time suggesting that the conversion of hematin to bilirubin occurs relatively slowly in contrast to the rapid conversion of hemoglobin to bilirubin

J F R

NEWS AND VIEWS

Dr Henry Poncher of Chicago led a symposium on Anemia in Childhood for presentation before the Pediatric section of the American Medical Academy in San Francisco in July. Dr Wolf Zuelzer of Detroit discussed the pathogenesis of anemia, Dr James M. Baty, Boston, the classification, Dr Carl Smith, New York, the diagnosis, and Dr Poncher, the treatment. The discussion of the symposium was opened by Dr C. V. Moore, St. Louis, and Dr M. M. Wintrobe, Salt Lake City.

Dr Louis B. Jaques, Department of Physiology, University of Toronto, has been appointed Professor and Head of the Department of Physiology, University of Saskatchewan, Saskatoon, Canada. The Medical School of the University has been reorganized and will from now on give a full four year course. Dr Jaques' main interests have been in blood coagulation. He has played an important part in the clarification of the mechanism of action of heparin and in the development of this drug for clinical use in collaboration with other members of the departments of physiology and surgery of the University of Toronto.

Dr George Cartwright, former associate of Dr M. M. Wintrobe of Utah, is in Shanghai at present with the United States Army. He has written a comprehensive article on the dietary factors involved in hematopoiesis, which will be published in one of the forthcoming issues of BLOOD.

Dr William Dameshek of Boston has received a grant from the United States Public Health Service for study of the Mediterranean target-oval cell syndromes and their possible relationships to sickle cell disease.

An International Rh and Hematological Conference is to be held in Dallas on November 15 in affiliation with the National Mexican Transfusion Congress which is to be held the following week in Mexico City. Some of the workers in this field who have accepted invitations to participate in the Dallas section of the conference are Doctor Race of England, Doctors Gonzales-Guzman and Uribe of Mexico City, and Doctors Philip Levine, Louis K. Diamond, and William Dameshek of Linden, New Jersey, and Boston, respectively. English-Spanish preprints are to be prepared, together with a 'crawling title' motion picture for projection simultaneously with the oral presentations.

The following European physicians have accepted posts as Contributing Editors to BLOOD:

England L. J. Witts, Oxford
 J. V. Dacie, London

<i>Scotland</i>	L J Davis, Glasgow
<i>France</i>	P Emile-Weill, Paris
<i>Belgium</i>	J Roskam, Liège
<i>Switzerland</i>	Karl Rohr, Zürich
<i>Holland</i>	J J Groen, Amsterdam
<i>Denmark</i>	E Meulengracht, Copenhagen
<i>Sweden</i>	J Waldenström, Upsala
<i>Turkey</i>	E Frank, Istanbul

Further additions to this list are expected in the near future

Dr Charles P Emerson, Jr, Thorndike Memorial Laboratory, joined the staff of the Evans Memorial Hospital on July 1st. He has been appointed Assistant Professor of Medicine at Boston University Medical School and is in charge of the blood bank. He is continuing research in hemolytic syndromes.

Dr Henri J Tagnon, Thorndike Memorial Laboratory, has been awarded a Senior National Research Council Fellowship beginning July 1, 1946. He is to work at the Memorial Hospital in New York.

Dr A H T Robb-Smith, Director of Pathology, Radcliffe Infirmary, Oxford, England, visited a number of American clinics last February. He mentioned that the Oxford group was planning an International Hematological Congress sometime in the future, probably in the summer of 1948. Will those who are interested or who have suggestions apropos the proposed Hematological Congress communicate with Dr Steven O Schwartz, 55 E Washington St, Chicago 2, Illinois.

WILLIAM WADDELL DUKE

NOTES ON THE MAN AND HIS WORK

BIOGRAPHICAL notes have much in common with clinical case reports. For one thing, they should be written only after intimate contact with the case in question, or they risk being superficial and disjointed. The writer never met



WILLIAM WADDELL DUKE

W W Duke in person. He has, however, become familiar with the man through his writings, from other men's accounts, and from correspondence. What is said here is based mostly on such objective, unintimate evidence.

Duke was 63 years old when he died in Kansas City on April 10, 1946. He was born in October 1882, in Lexington, Missouri, graduated in 1904 from Yale,

where he majored in biology, and from Johns Hopkins Medical School in 1908. He then won an appointment as Medical House Officer in the Massachusetts General Hospital, where he remained for one and a half years. The following few months were spent as a resident in tuberculous sanatoriums. His investigative bent must have drawn him back to Johns Hopkins, where he became associated with William H. Howell in 1910.

Howell at the time was engaged in studying the physiology of the heart beat. Duke collaborated with him in several papers dealing with the role of calcium and potassium in the nervous regulation of heart action.

At Johns Hopkins, Duke came in contact with William H. Welch and G. H. Whipple in the Hunterian Laboratory of Experimental Pathology. In one of his letters, Duke acknowledges the support that Popsy Welch gave him in getting his work accepted and published. Much of the encouragement he received at the time came from Welch, whose interest in platelets began during his work on experimental thrombosis.

Duke's own interest in blood platelets probably dated back to his years at the Massachusetts General Hospital and his contacts with J. Homer Wright. Some of the patients with thrombopenic purpura quoted in Duke's papers had been observed at the Massachusetts General Hospital, and others had been collected during Duke's visit to the European clinics of Berlin, Vienna (Professor Riehl), and Tübingen (Professor Rhomberg).

Duke did most of his work on platelets and hemorrhagic disease between 1908 and 1915. It was in the early part of this period that he came under the influence of J. H. Wright, when Wright was occupied with the demonstration of the origin of platelets from megakaryocytes. Wright's first publication in 1906 (*Boston Medical and Surgical Journal* 154: 643) had not made a favorable impression in some quarters, owing perhaps to poor reproduction of his illustrations. In 1910, Wright published his experiments in greater detail and illustrated them with colored plates which did full justice to his views. Duke had great admiration for Wright and said in one of his letters:

With reference to J. H. Wright I look upon him as one of the most brilliant pathologists I have ever known. He did the laboratory work on the platelet counting method published under the name of Wright and Kinnicut, I helped with it but the idea was entirely Wright's. I graduated from M. G. H. in the midst of this work.

To Duke perhaps belongs the credit for the conclusive demonstration of the role of the platelets in hemostasis. This he did by devising the test known as the bleeding time. Up to the time of Duke, attempts to connect hemorrhagic tendencies with a diminution in the number of blood platelets had rested on the presence or absence of purpura, or bleeding from the mucous membranes. Duke showed how misleading this correlation could be, since the purpura depended much on the degree of physical activity or the trauma undergone by the patient *before* being observed.

When Duke heard of patients who supposedly had normal bleeding times even though the platelet count was low, he was ready to inquire how the determination

was carried out. To his chagrin he frequently learned that what was called the bleeding time was often obtained from a puncture made with a sewing needle or similar tools.

In one of his letters, Duke states

I am sorry to say that I am usually misquoted in relation to the method of obtaining the bleeding time. It should be obtained in humans from the lobe of the ear by making a relatively large cut and blot the blood up at half minute intervals. If this is not done and the cut in the ear is too small, blood may collect on the ear, clot and dry and give the impression that the hemorrhage has been stopped. He realized the limitations in the clinical significance of his test, but felt strongly that when there was a reduction in platelets or in fibrinogen the bleeding time would be altered.

After 1915, Duke published comparatively little on the subject of his original interests. His last work on blood platelets was done after his return to Kansas City, where he was to remain the rest of his life. In his last paper on the subject (J. A. M. A. 62:1600, 1915) indications are already present of his rising interest in anaphylaxis and other forms of hypersensitiveness. The relation of platelets to the manifestations of hypersensitiveness was beginning to draw his attention. From 1915 on, Duke was occupied in the study of allergy. He introduced the concept of physical allergy and stressed the part played by cold, heat, and physical effort in conditioning abnormal tissue reactions.

Duke had a restless, scintillating mind which must have been often tempted to run into unbridled speculation. His style of writing was simple and direct. His experiments seemed to be neatly laid out and the results were expressed clearly, using factually accurate charts drawn by himself.

Generally speaking, his conclusions were held well within the evidence. These qualities lent his papers a certain strength which perhaps accounted for their general acceptance. They were, and are still, widely quoted in the world's literature. Duke was responsible, perhaps more than any other contemporary author, for strengthening the concept of the importance of platelets in disorders of hemostasis.

LEANDRO M. TOCANTINS, M. D.

BOOK REVIEWS

The Vitamins in Medicine By FRANKLIN BICKNELL AND FREDERICK PRESCOTT Grune & Stratton, Inc., New York 2nd edition Pp 928 \$12.00

During the last 30 years, an ever increasing amount of research has been devoted to the study of vitamins. This knowledge is spread throughout the literature of the last 3 decades and the magnitude of the work makes it almost impossible for clinicians and others to keep abreast of the subject.

Since the first edition of this book appeared in December 1942 further advances in knowledge of the vitamins have been made especially concerning the B complex, these have been suitably discussed in largely rewritten and expanded chapters devoted to these subjects. A short chapter has been added on the essential unsaturated fatty acids and minor soluble vitamins. The tables giving the vitamin content of foods have also been revised.

The major vitamins are considered under the following headings: history, chemistry, food sources, physiology, pharmacology, human requirements in health and disease, deficiency diseases, methods for detecting vitamin deficiencies and therapeutic uses. For the sake of simplicity, deficiency diseases are discussed under separate vitamins: e.g. beriberi under Vitamin B, scurvy under Vitamin C and so on. This may give the impression that such diseases are due to the lack of a single vitamin but, as the authors caution, deficiency diseases are usually due to multiple deficiencies and it would be extremely difficult to devise a diet lacking in just one factor.

The authors (who are British) state that the majority of the public are apathetic towards the problem (of vitamin deficiency) and are content to spend their lives in that shadowed region between good health and frank illness. This may be true for England, but the millions of dollars spent yearly for the advertising and purchase of the various vitamins in this country vigorously belie this statement. It might more aptly be said that the public is completely confused by the problem, reflecting the conflicting opinion and often confused knowledge of the subject by physicians and their co-workers in the nutritional field. The authors have correlated up-to-date information on the chemistry, physiology, nutritional importance and clinical uses of the vitamins with emphasis laid upon the clinical aspect of the subject. Each subject is followed by an exhaustive and well selected bibliography of the subject. References to original papers number nearly 4500 and the text is amply illustrated with 208 equally well chosen descriptive and beautifully printed illustrations.

The general excellence of the book, the remarkable bibliography and the timeliness and importance of the subject make this a work that can be unreservedly recommended as a must addition to the library of every clinician and nutritionist.

PRESERVATION OF NORMAL HUMAN PLASMA IN THE LIQUID STATE

V CLINICAL, CHEMICAL, AND PHYSICOCHEMICAL STUDIES DURING THREE YEARS OF STORAGE AT ROOM TEMPERATURE*

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NOTWITHSTANDING the fact that liquid plasma is a good bacterial culture medium (even when containing bacteriostatic agents in clinically nontoxic concentrations), it remains a popular form for preservation because its preparation is simple and economical and because it is the only form in which the plasma is instantly available. It has been shown by many investigators, first by Kartashevsky and Filatov in Russia in 1934,¹ that plasma may be preserved in the liquid state for many months and administered with clinical safety. Recent workers in this field have emphasized the need for meticulous asepsis in the technics used in drawing blood and preparing plasma.^{2,3} A closed-vacuum system has been advocated by several investigators^{4,5} and has much to recommend it. In this regard, it is pertinent to call attention to a recent report on the sterilization of citrated plasma by filtration,⁶ a procedure which may considerably increase the safety of a liquid plasma program.

Previous papers from this Institute^{3,7,8} have presented the results of clinical, physiological, and chemical studies on plasma preserved in the liquid state at room temperature for two years. The conclusion of these studies was that when liquid plasma is properly prepared it may after such an extended period of storage be administered to patients with safety and benefit despite the inactivation within six months of certain labile constituents and a slow increase in nonprotein and alpha-amino nitrogen† content. It was pointed out in one of the papers⁸ that gross cleavages in the plasma proteins might have occurred without significant increase in nonprotein nitrogen as long as the products of such cleavage remained large enough to be insoluble in the usual protein precipitant. For this reason physicochemical methods were employed in order to demonstrate whether or not such cleavages were taking place. It is the purpose of the present communication to report the results of osmometric, viscosimetric, and electrophoretic studies on plasma stored for three years in the liquid state and to summarize the chemical and clinical observations to date. The practical application of these studies is clear-cut, they form

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‡ The term alpha amino nitrogen is used to designate the result of the gasometric determination of free amino acids in blood filtrates by the ninhydrin carbon dioxide method.

a reasonable basis for the determination of the expiration date of plasma preserved in the liquid state

MATERIALS AND METHODS

Samples—Studies were continued on samples from the same eleven pools of plasma prepared by the Blood and Plasma Department of the Naval Medical School, upon which the previous reports⁸ have been based. Inasmuch as serial samples had been removed from these pools and the effect of such sampling was unknown, lots from which no previous samples had been removed were obtained from four pools of approximately the same age as the original eleven. All sampling was done aseptically.

Chemical Studies—Nonprotein nitrogen was determined by the manometric micro Kjeldahl technique⁹ using tungstic acid and 2.5 per cent trichloroacetic acid as the protein precipitants and the interpretation of these precipitants was that proposed by Hiller and Van Slyke.¹⁰ Alpha amino nitrogen was determined gasometrically by the ninhydrin method.¹¹

Osmometric Studies—Samples of fresh and stored plasma were dialyzed in Visking casing in the cold for four days against a reference fluid consisting of buffered saline and then placed in an osmometer constructed on the basis of the Davis¹ modification of the Hepp instrument¹² using parlodion membranes with the plasma above and fresh reference fluid below the membrane. These studies were made at room temperature.

Viscosimetric Studies—A Cannon Fenske-Ostwald viscosimeter of the nonviscous type designed to reduce kinetic energy and surface tension corrections¹⁴ was used. All samples were passed through a sintered glass filter after which aliquots were taken for protein analysis, density measurement, glass electrode pH determination and viscosity measurements. All viscosity measurements were made in a water bath at $37.8 \pm 0.1^\circ \text{C}$.

Electrophoretic Studies—These were performed by Drs. Laura E. Krejci, Lucile Sweeney and Edward B. Sanigar of the Biochemical Research Foundation of the Franklin Institute, Newark, Delaware, and have been reported upon in detail elsewhere.¹⁵ They were carried out by the Tiselius method¹⁶ photographs being taken by both the Longworth¹ and Svensson techniques.¹⁵

Clinical Studies—The questionnaire technique previously described² was used. Three thousand three hundred and eighty-four questionnaires which had been filled out following the administration of plasma stored in the liquid state for more than a year were analyzed. Fifteen hundred questionnaires on the administration of commercially prepared dried plasma served as controls.

RESULTS

Chemical Findings—The chemical data which have been accumulated during the past two years have been arbitrarily divided into three analysis periods (table 1), corresponding roughly to the second year of storage, and the beginning and end of the third year, respectively. The changes in alpha-amino nitrogen (N) concentration (table 2, fig. 1) indicate a rise from 5 mg. N per 100 ml. in fresh plasma to about 12 mg. N per 100 ml. during the second year of storage and not much further increase during the third year. The last four samples listed in table 2 are those mentioned above as not having been previously analyzed. It will be observed that the results of their analysis are within the range of the rest of the group and that no effect can be attributed to multiple aseptic samplings.

The data on the nonprotein nitrogen concentration as determined from the 2.5 per cent trichloroacetic acid filtrate (table 3, fig. 2) show a somewhat steadier increase on storage than do the data on nonprotein nitrogen concentration as determined from the tungstic acid filtrate (table 4, fig. 3). It will be recalled that the filtrate from the former precipitant includes polypeptides as well as amino acids, whereas in the latter filtrate polypeptides are absent but amino acids are still included.¹⁰ The stabilization of the nitrogen content of the tungstic acid filtrate

during the third year of storage corroborates therefore the stabilization of the alpha-amino nitrogen during the same period. The difference between the nitrogen content of the trichloroacetic acid and the tungstic acid filtrate has been termed the polypeptide index¹⁹ and appears to be a relatively accurate indication of the polypeptide content of a material. As might be expected from the progressive increase in the nitrogen content of the trichloroacetic acid filtrate and stabilization of the tungstic acid filtrate nitrogen, the polypeptide index also rises

TABLE 1—*Analysis Periods for Chemical Studies of Stored Plasma*

Period I (11 samples)			Period II (15 samples)			Period III (15 samples)		
Age (months)			Age (months)			Age (months)		
Min	Max	Av	Min	Max	Av	Min	Max	Av
15	24	18	24	35	28	31	39	34

TABLE 2—*Alpha amino Nitrogen Concentration in Pooled Human Plasma at Three Analysis Periods*

Lot no	Period I		Period II		Period III	
	Age (mo)	Alpha amino nitrogen	Age (mo)	Alpha amino nitrogen	Age (mo)	Alpha amino nitrogen
		mg %/100 ml		mg %/100 ml		mg %/100 ml
154 4	24	12.1	33	12.8	39	12.4
257 3	20	11.9	29	13.0	36	12.7
259 2	20	14.0	29	15.5	36	14.2
289 3	20	11.5	29	12.4	36	11.8
295 6	19	11.8	28	12.7	36	12.0
383 1	18	10.2	27	11.0	34	10.5
781 3	15	11.0	24	12.3	31	11.8
783-1	15	10.3	24	11.3	31	10.5
852 5	15	9.9	24	11.6	31	11.1
853 7	15	10.2	24	11.6	31	11.5
856-3	15	9.0	24	10.0	31	9.9
197 5			35	11.9	38	12.1
290-4			32	13.3	36	12.8
291 4			32	11.8	36	11.2
292 4			32	10.8	36	10.6
Average	18	11.1	28	12.1	34	11.7

steadily during the three years of storage (table 5, fig. 4). To recapitulate all these findings a summary table has been prepared which includes data on fresh controls (table 6).

Osmometric Findings—Stored plasma is slightly but significantly more effective osmotically than fresh plasma (table 7).

Viscosimetric Findings—The glucose and citrate which had been added to the native plasma in the course of its preparation complicated comparative measurements, and control studies had to be made on water, saline, citrated saline con-

TABLE 3 — *Nonprotein Nitrogen of 2.5% Trichloroacetic Acid Filtrates of Pooled Human Plasma at Three Analysis Periods*

Lot no	Period I		Period II		Period III	
	Age (mo)	$\frac{\text{NPN}}{2.5\% \text{ T.C.A.}}$	Age (mo)	$\frac{\text{NPN}}{2.5\% \text{ T.C.A.}}$	Age (mo)	$\frac{\text{NPN}}{2.5\% \text{ T.C.A.}}$
		mg \100 ml		mg \100 ml		mg \100 ml
154-4	24	44.8	33	56.6	39	56.9
257-3	20	41.2	29	57.0	36	56.5
259-2	20	43.2	29	62.0	36	58.2
289-3	20	47.7	29	55.5	36	57.4
295-6	19	49.1	28	56.4	36	60.6
383-1	18	35.7	27	50.1	34	61.2
781-3	15	44.9	24	51.2	31	62.7
783-1	15	40.4	24	48.8	31	56.6
852-5	15	44.2	24	46.5	31	58.9
853-7	15	35.0	24	47.5	31	57.1
856-3	15	41.5	24	44.8	31	55.5
197-5			35	50.7	38	56.3
290-4			32	49.3	36	56.3
291-4			32	49.8	36	58.3
292-4			32	47.7	36	61.4
Average	18	42.5	28	51.6	34	58.3

TABLE 4 — *Nonprotein Nitrogen of Tungstic Acid Filtrates of Pooled Human Plasma at Three Analysis Periods*

Lot no	Period I		Period II		Period III	
	Age (mo)	$\frac{\text{NPN}}{\text{T.A.}}$	Age (mo)	$\frac{\text{NPN}}{\text{T.A.}}$	Age (mo)	$\frac{\text{NPN}}{\text{T.A.}}$
		mg \100 ml		mg \100 ml		mg \100 ml
154-4	24	35.3	33	39.2	39	41.0
257-3	20	32.5	29	41.3	36	39.4
259-2	20	40.0	29	41.4	36	40.8
289-3	20	43.7	29	43.9	36	39.0
295-6	19	36.9	28	43.6	36	40.3
383-1	18	31.6	27	35.7	34	36.1
781-3	15	40.4	24	45.4	31	39.7
783-1	15	35.6	24	39.2	31	37.8
852-5	15	36.3	24	36.8	31	36.5
853-7	15	35.2	24	37.1	31	37.1
856-3	15	34.4	24	36.0	31	32.3
197-5			35	38.3	38	37.7
290-4			32	39.9	36	35.0
291-4			32	37.0	36	36.7
292-4			32	36.1	36	37.5
Average	18	36.5	28	39.4	34	37.8

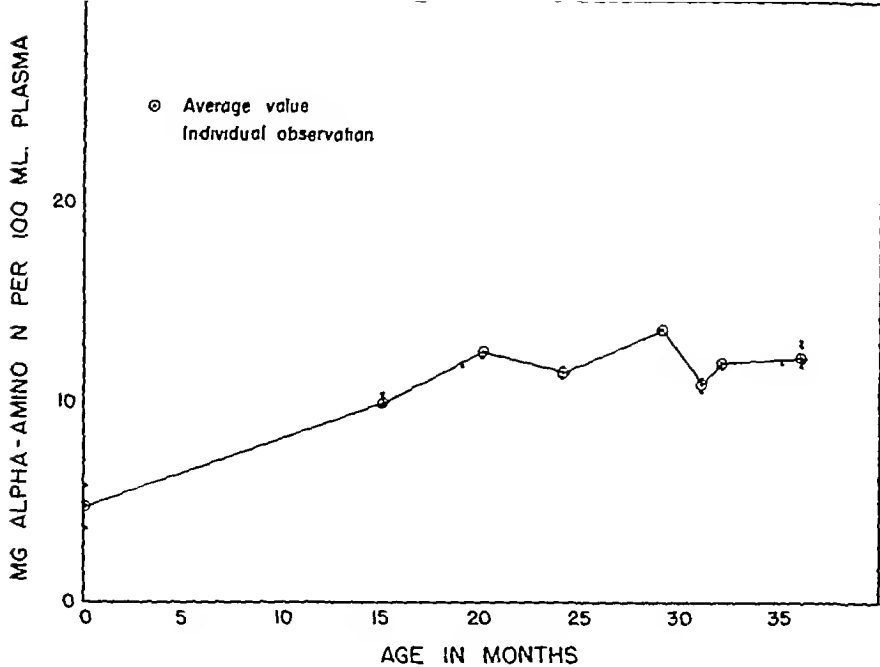


FIG 1 THE EFFECT OF STORAGE OF LIQUID PLASMA FOR PERIODS UP TO 39 MONTHS ON THE ALPHA-AMINO NITROGEN OF THE PICRIC ACID FILTRATE

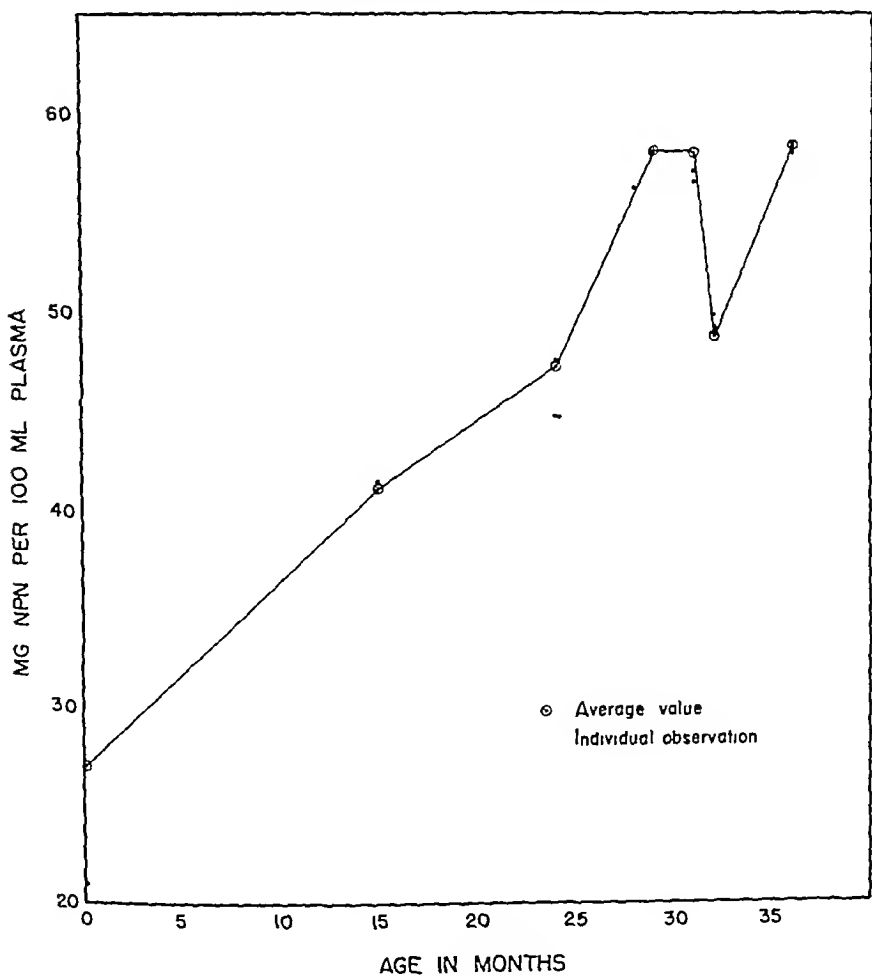


FIG 2 THE EFFECT OF STORAGE OF LIQUID PLASMA FOR PERIODS UP TO 39 MONTHS ON THE N P N CONCENTRATION OF THE 2.5 PER CENT TRICHLORACETIC ACID FILTRATE

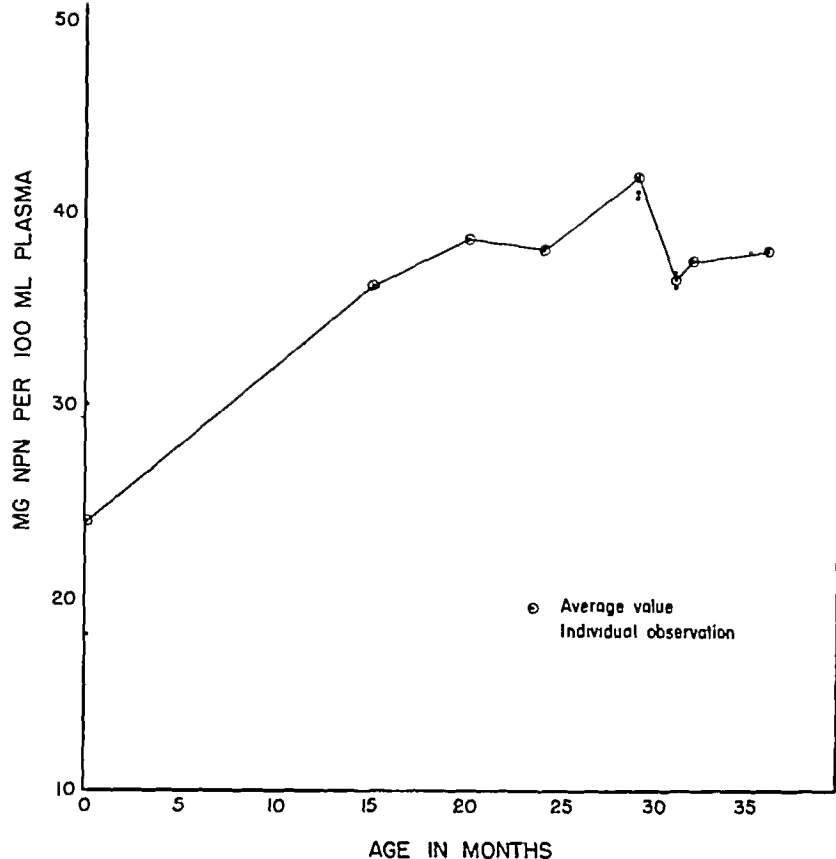


FIG 3 THE EFFECT OF STORAGE OF LIQUID PLASMA FOR PERIODS UP TO 39 MONTHS ON THE N P N CONCENTRATION OF THE TUNGSTIC ACID FILTRATE

TABLE 5 —Polypeptide Index of Pooled Human Plasma at Three Analysis Periods

Lot no	Period I		Period II		Period III	
	Age (mo)	Peptide index	Age (mo)	Peptide index	Age (mo)	Peptide index
		mg N/100 ml		mg N/100 ml		mg N/100 ml
154-4	24	9.5	33	17.4	39	15.9
257-3	20	8.7	29	15.7	36	17.1
259-2	20	3.2	29	20.6	36	17.4
289-3	20	4.0	29	11.6	36	18.4
295-6	19	12.2	28	12.8	36	20.3
383-1	18	4.1	27	14.4	34	25.1
781-3	15	4.5	24	5.8	31	23.0
783-1	15	4.8	24	9.6	31	18.8
852-5	15	7.9	24	9.7	31	22.4
853-7	15	0	24	10.4	31	20.0
856-3	15	7.1	24	8.8	31	23.2
197-5			35	12.4	38	18.6
290-4			32	9.4	36	21.3
291-4			32	12.8	36	21.6
292-4			32	11.6	36	23.9
Average	18	6.0	28	12.2	34	20.5

TABLE 6—Summary of Chemical Studies on Pooled Human Plasma at Three Analysis Periods

Nitrogen components	Fresh (Con trol) (1-7 days)		Stored (Period I) Av age 18 months			Stored (Period II) Av age 28 months			Stored (Period III) Av age 34 months			
	mg nitrogen per 100 ml plasma											
	min	max	min	max	av	min	max	av	min	max	av	
Alpha amino nitrogen	4	6	9	14	11	10	15	12	9	14	11	
Nonprotein nitrogen												
Filtrates 2.5% CCl ₃ COOH	21	33	35	49	42	54	62	51	55	62	58	
Tungstic acid	18	30	31	43	36	35	45	39	32	41	37	
Polypeptide index NPN 2.5% CCl ₃ COOH —												
NPN Tungstic acid	3	8	0	12	6	8	10	6	12	15	10	

TABLE 7—Osmometric Studies on Dialyzed Plasma

Type of plasma	Colloid Osmotic Pressure/Concentration mm water/Gm protein/100 ml	
	Mean	Standard Deviation
Fresh (22 specimens)	44.6	±0.6
Stored (over 2 years) (12 specimens)	48.6	±0.4

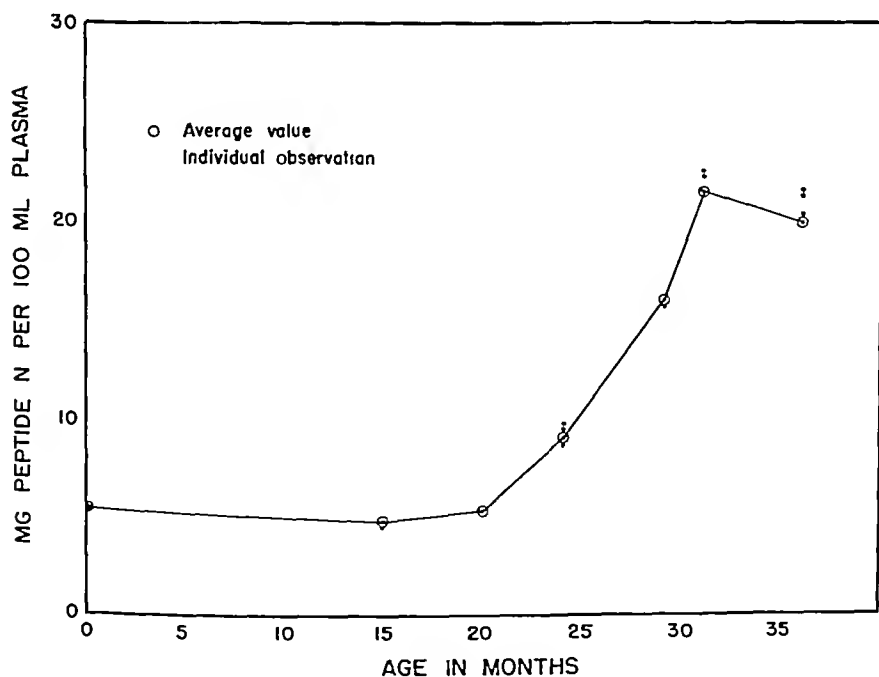


FIG 4 THE EFFECT OF STORAGE OF LIQUID PLASMA FOR PERIODS UP TO 39 MONTHS ON THE PEPTIDE INDEX NITROGEN OF THE PLASMA

TABLE S—Viscosity Measurements on Fresh Pooled Plasma and Stored Pooled Plasma
Measurements at $37.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$

Solution	Average glucose concentration	No of samples	Age	Av protein concentration Gm/100 ml	pH	Viscosity (a) centipoises	Relative viscosity to		
			months				H ₂ O	0.1% NaCl	3% Na Citrate 5.0% Glucose 0.15 M NaCl
Citrated saline (0.15 M NaCl 3% Na Citrate)	5%				6.9	783	1.15	1.1	
Fresh citrated plasma (3% Na Citrate)	0	1	(1 day)	5.45		1.114	1.63	1.59	
	0*	1		5.5			1.68	1.60	
	5%	6	1	5.35	7.1	1.2-5	1.79	1.76	1.56
Stored citrated plasma (3% Na Citrate)	5%	3	37	4.73	6.7	1.238	1.81	1.78	1.58
	5%	1	38	4.89	6.8	1.235	1.81	1.78	1.58
	5%	1	40	4.55	6.8	1.361	1.99	1.95	1.74
	5%	1	41	4.88	6.5	1.60	1.84	1.81	1.61

* Comparative data (measured at 37°C) taken from Strumia M, personal communication

† Actual protein concentration, Gm/100 ml of solution uncorrected for citrate and glucose dilution

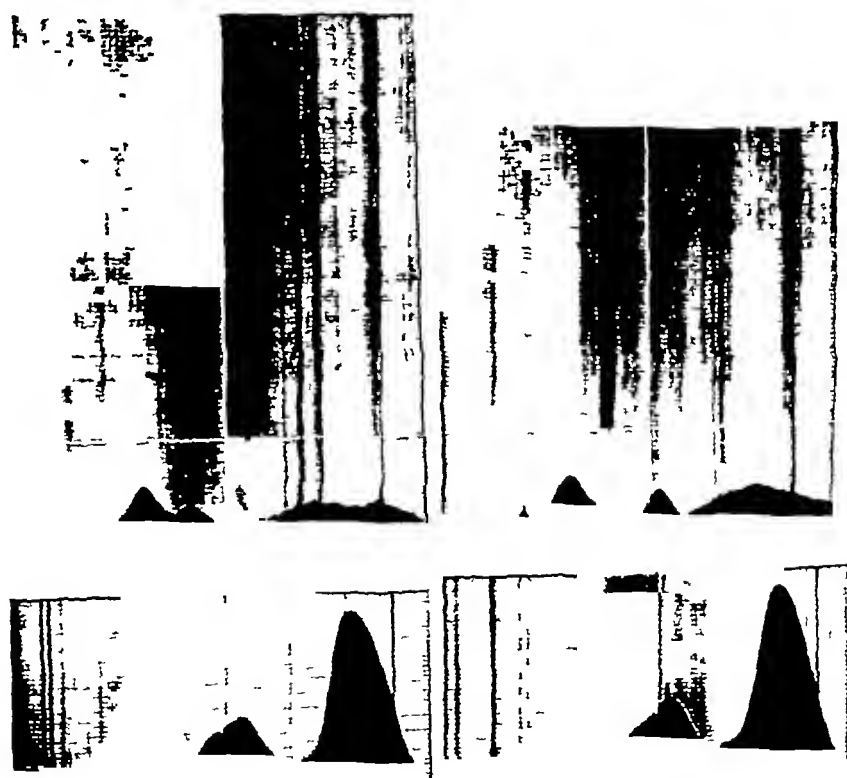


FIG 5 ELECTROPHORETIC PATTERNS OF FRESH PLASMA (LONOSWORTH DIAGRAMS)

Upper diagrams, rising boundaries. Lower diagrams, descending boundaries. To the left 5% glucose added, photographs taken after passage of 934 coulombs (approximately 4.5 hours at 27 ma plus 11.5 hours at 12 ma). To the right, dialyzed glucose-free, photographs taken after passage of 880 coulombs (approximately 5 hours at 40 ma plus 16.5 hours at 2.7 ma). The dark vertical band above the fibrinogen boundary in each of the lower diagrams was caused by precipitation of protein during electrophoresis.

taining 5 per cent glucose, and fresh citrated plasma with and without glucose. In order to facilitate the interpretation of the data obtained, all the pertinent measurements have been tabulated (table 8). Included in the table is the only viscosity measurement on citrated plasma by another investigator that we have been able to find.²⁰ His measurement checks with our data. The results indicate that three year old plasma is slightly more viscous than fresh plasma but that the difference is of no practical significance.

Electrophoretic Findings—Krejci, Sweeny, and Sanigar have presented the Svenson diagrams elsewhere.¹⁵ The Longworth diagrams are presented here (figs. 5 and 6). Patterns were made of fresh plasma containing glucose (fig. 5), fresh



FIG. 6. ELECTROPHORETIC PATTERNS OF THREE YEAR OLD PLASMA
290-4 (LONGWORTH DIAGRAMS)

Upper diagrams, rising boundaries. Lower diagrams, descending boundaries. To the left in 5% glucose, photographs taken after passage of 756 coulombs (approximately 6 hours at 27 ma. plus 16 hours at 3 ma.) To the right dialyzed glucose free, photographs taken after passage of 659 coulombs (approximately 4.5 hours at 40 ma. plus 16 hours at 0.2 ma.) The dark vertical band above alpha and beta boundaries in each of the upper diagrams was caused by precipitation of protein during electrophoresis.

plasma dialyzed glucose-free (fig. 5), three year old plasma containing glucose (fig. 6), and three year old plasma dialyzed glucose free (fig. 6). The patterns show that at the end of three years' storage there is a large increase in components with the mobilities of alpha globulin and albumin at the expense of all the gamma globulin, all the fibrinogen, and part of the beta globulin. Equally striking was the observation that the mobilities of the stored plasma were 20 to 25 per cent greater than the fresh plasma and that the boundaries were broadened. In an attempt to explain these findings, Krejci, Sweeny, and Sanigar¹⁵ subjected fresh plasma to heat, and this was found to cause a similar increase of the alpha globulin but at the expense of different plasma constituents and without increase of the mobilities or broadening of the boundaries.

DISCUSSION

The problem in the interpretation of these data is the determination of their clinical significance. This question can, of course, be answered best by a careful appraisal of the clinical results obtained by the administration of liquid plasma stored for various lengths of time. Inasmuch as until recently the expiration date for liquid plasma specified by the National Institute of Health was one year and the present expiration date is eighteen months, administration of older plasma has proceeded slowly. To date, questionnaires on some 3,000 administrations of plasma

TABLE 9 — *Age Distribution of Stored Liquid Plasma Series*

Age of Plasma (months)	Number of Administrations
12-17	1663
18-23	829
24-29	569
30-35	284
36-40	39
Total	3384

Mean age 19 6 mos.
 Standard deviation of the age distribution 6 3 mos

TABLE 10 — *Comparison of Percentage and Type of Untoward Reactions in 1500 Administrations of Dried Plasma and 3384 Administrations of Stored Liquid Plasma*

Type of Reaction	Dried Plasma per cent	Stored Liquid Plasma per cent
Mild pyrogenic	1 7	0 08
Mild urticarial	0 9	0 03
Mild miscellaneous	0 5	0 30
Moderate pyrogenic	1 1	0 15
Moderate urticarial	0 7	0 06
Moderate miscellaneous	0 0	0 15
Severe pyrogenic	0 2	0 06
Severe urticarial	0 1	0 00
Total	5 2	0 83

more than a year old have been analyzed (table 9). The untoward reactions following these administrations have been contrasted (table 10) with those following administration of dried plasma, the latter presumably being comparable to fresh plasma. Possible reasons for the striking decrease of reactions with plasma storage have been discussed previously.³

The stored plasma was reported by the medical officers administering it to have been as therapeutically effective as fresh plasma. These satisfactory results are not incomprehensible when it is considered that most plasma is administered because of its colloid content and that the colloid content of stored plasma is to all

intent unchanged. The maximal increase to date in the nitrogen content of the trichloroacetic acid filtrate represents hydrolysis to nonprotein size of only between 3 and 4 per cent of the original protein. It is evident, however, that from the physicochemical point of view more striking changes have occurred on storage. These are most dramatic in the electrophoretic analyses but are also significant in the osmometric and viscosimetric measurements. The results of the latter two determinations would appear to have little influence on the decision to permit the widespread clinical use of liquid plasma stored this length of time. They indicate only that the plasma remains osmotically active and is of a viscosity which is practical for intravenous administration. It is interesting to speculate on the possible reasons for the increase in viscosity which took place on storage. Every other measurement we have made is explained by slight proteolysis. Proteolysis of protein, however, is generally stated to be accompanied by a decrease in viscosity.²¹ Denaturation, such as precedes coagulation, on the other hand, is stated to be associated with a viscosity increase.²² It appears, therefore, that in this case the viscosity is affected more by the slight denaturation which has taken place than by the slight hydrolysis.

The clinical implications of the electrophoretic diagrams are important. The complete disappearance of the gamma globulin component is in accord with previous physiological studies⁷ which have pointed out that blood coagulation factors and complement deteriorate rapidly on storage. Since it is now known that most antibodies are included in the gamma globulin fraction,²³ it is evident that stored liquid plasma must never be used for immune properties or to supply blood coagulation factors in patients with hemorrhagic diatheses. Even in patients with decreased blood volume who are receiving plasma for its colloid content, it would appear unwise to administer large amounts of plasma devoid of coagulation properties or complementary activity. By overenthusiastic use of stored liquid plasma the patient's globulin and prothrombin content may conceivably fall as a result of dilution in a way comparable to that which has already been described for albumin.²⁴

To summarize our clinical interpretation of these results, it may be stated that liquid plasma stored for periods up to three years would appear to be an adequate therapeutic agent to supply colloid of human origin. It is believed that except for the fact that stored liquid plasma is relatively isotonic, whereas human serum albumin as supplied at present is hypertonic, the two therapeutic agents are quite similar in their clinical indications and precautions. Both are preserved in the liquid state at room temperature. Neither is known to supply much except a human colloid. Neither should be used excessively.

Finally, it must be emphasized again that liquid plasma even when containing bacteriostatic agents may permit bacterial growth. Accidental contaminants during preparation may multiply to dangerous proportions during preservation for even short periods. The technique of drawing blood and plasma preparation should be with closed systems and meticulously aseptic. Bacteriologic control must be scrupulous throughout.

SUMMARY

1 During preservation of human plasma in the liquid state at room temperature for three years, the alpha-amino nitrogen and the nitrogen content of the tungstic acid filtrate rise slowly until about two years have elapsed, after which time these levels do not increase significantly

2 During the entire period of storage the nitrogen contents of the trichloroacetic acid filtrate and the polypeptide index increase progressively. The actual increase represents hydrolysis to nonprotein size of 3 to 4 per cent of the original protein

3 The colloid osmotic pressure of stored plasma is slightly but significantly greater than that of fresh plasma

4 The viscosity of stored plasma is slightly but significantly greater than that of fresh plasma

5 The electrophoretic patterns of stored plasma show increases of alpha globulin and albumin concentration, complete disappearance of gamma globulin (containing immune properties) and fibrinogen, and some reduction of beta globulin concentration as compared to fresh plasma

6 Analysis of 3,384 questionnaires completed after administration of liquid plasma more than a year old indicates that the transfusion of such plasma continues to be safe and beneficial up to at least three years of storage. The untoward reaction rate following these administrations was significantly less than that following a comparable series of 1500 administrations of commercially prepared dried plasma

CONCLUSIONS

1 The chemical and physicochemical changes observed in the systems studied in properly prepared plasma stored in the liquid state at room temperature are of no more clinical significance at the end of three years storage than at the end of eighteen months, the present National Institute of Health expiration date

2 Because at the end of six months storage, liquid plasma is devoid of blood coagulation factors, complementary activity, and probably other antibodies, it should not be administered to patients with either hemorrhagic diatheses or infections and who require these components specifically. Hence, it should be used chiefly for its colloid content and should be subject to the same precautions regarding excessive dosage as is human serum albumin

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Commander H R Evans, (HC), U S N , and Lieutenant Commander M T Sproul, H(W), U S N R

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PLATELET COUNTS AND PLATELET FUNCTION

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With the technical assistance of EDITH MILLS, M.A.

INTRODUCTION

THE frequent association of thrombocytopenia with increased capillary fragility, prolonged bleeding time, and diminished clot retraction is well known. However, there are few reports showing the exact nature of this relationship. The principal reason for the lack of such data is the unreliability of the platelet count.

Under ordinary circumstances little attention is given to the technical difficulties involved in making an accurate count of the blood platelets. The values obtained are often referred to textbook standards. The normal range is usually stated to be 200,000 to 400,000 per cubic millimeter, and the critical level of pathologic significance is variously given as 50,000 to 75,000 per cubic millimeter. In actual practice it is found that the normal and critical levels of the platelet count vary depending upon the methods employed and upon individual variations between different technicians. In order to insure reliability in the platelet count it is necessary that it be done by an experienced technician and that it be evaluated in terms of that individual's data regarding standards of normality. That this ideal has seldom been attained is obvious from a review of the literature relating to the clinical significance of the blood platelet.

The present investigation was undertaken in order to determine the significance of reductions in the platelet count in terms of well-established normal standards and to define the relationships which exist between the platelet count and the results of tests of the bleeding time, coagulation time, clot retraction, and capillary fragility. The only previous comprehensive study of this subject that we have encountered is that of Tocantins,¹ which deals with experimental thrombocytopenic purpura in dogs.

PLAN OF STUDY

Observations of the bleeding time, coagulation time, capillary fragility, clot retraction, and platelet count were made on each of 64 normal subjects, and on 404 patients suffering from various diseases. Additional observations were made in selected cases. All of the platelet counts were done by one experienced technician (E.M.) and the remaining tests were done under the direct supervision of P.M.A. Each set of observations was done on the same day and usually at the same sitting.

The assembled data, collected over a period of five years (1938-43), were sub-

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jected to statistical analysis by J H ²³ The technics employed and the results of the analyses will be considered under their appropriate headings The normal values for each of the tests are given in table 1 The statistical relationships between the platelet count and the results of the various tests employed to determine platelet function are given in table 2

METHODS

Platelet Count A separate set of blood-counting apparatus was used exclusively for the platelet counts Capillary blood was obtained from a puncture wound in the lobe of the ear, care being taken to obtain a free flow of blood A red blood cell pipet was freshly rinsed with Rees and Ecker diluting fluid,⁴ blood was drawn to

TABLE 1.—Normal Values Based on a Single Series of Observations on Each of 64 Subjects

	Mean	Standard error of mean	Standard deviation	Normal range
Platelet count per cu mm	409,000	±8,500	±68,000	273,000 to 545,000
Fluid volume of clot in per cent of total specimen	7.9	±0.75	±6.0	—4.1 to 19.9
Bleeding time in minutes	3.2	±0.20	±1.6	0 to 6.4
Capillary fragility in numbers of petechiae				0 to 10
Coagulation time in minutes	8.9	±0.34	±2.7	3.5 to 14.3

TABLE 2.—Coefficients of Correlation and Association between the Platelet Count and Tests of the Fluid Volume of the Clot, Bleeding Time, Capillary Fragility and Coagulation Time The Results are Based on 631 Series of Observations Made on 404 Patients Suffering from Various Diseases

	Coefficient of correlation	Coefficient of association (Yule's Q)
Fluid volume of clot	—0.55 ± 0.03	0.88 ± 0.01
Bleeding time	—0.59 ± 0.02	0.87 ± 0.03
Capillary fragility	—0.38 ± 0.03	0.70 ± 0.05
Coagulation time	—0.21 ± 0.04	0.20 ± 0.16

the 0.5 mark, and the pipet was filled with diluting fluid and shaken for 3 minutes After the counting chamber was filled, it was allowed to stand for exactly 10 minutes The number of platelets seen in five small ruled squares of the chamber (the same areas used in the red blood cell count) was multiplied by 10,000 in order to determine the platelet count In all cases at least two chambers were counted, and in some cases as many as six chambers were counted and the results were averaged

Clot Retraction The fluid volume of the clot* was measured by a method described by Aggeler, Lucia, and Hamlin ^{5,6} Five cubic centimeters of venous blood were placed in a 15 cc graduated centrifuge tube A cork, fitted with a copper wire bent in the form of a hook, was adjusted so that the hook was immersed in the upper

*Formerly called the extracorporeal volume of the clot

layers of the blood. After coagulation of the blood had occurred, the tube was placed in a water bath at 37° C for 1 hour. The total volume (T) of the specimen was recorded and the retracted clot was removed by means of the wire hook. The residual volume of serum (S) was then recorded, and the volume occupied by the clot (C) was found by subtracting the serum volume from the total volume. The per cent of the specimen occupied by the clot was then calculated by dividing the clot volume by the total volume of the specimen and multiplying by 100. The volume (PCV) occupied by the entire packed cellular elements of the blood (RBC, WBC, and platelets) was determined on a separate specimen by Wintrobe's method.⁷ The volume of the entire specimen occupied by fluid occluded within the clot (FV) was found by subtracting the packed cell volume from the clot volume per cent. The entire calculation can be expressed by the following formulae:

$$T - S = C$$

$$\frac{C}{T} \times 100 - PCV = FV \text{ (fluid volume of clot)}$$

Bleeding Time The Ivy method was employed.⁸ A pressure of 40 mm Hg was maintained on the arm using a sphygmomanometer. A puncture wound 2 mm in length and depth was made in the relatively avascular area of the forearm, just distal to the antecubital fossa. The escaping blood was absorbed on gauze pledgets and the bleeding time was recorded to the nearest one-half minute.

Capillary Fragility The Dalldorf negative pressure method was employed.⁹ A pressure of 200 mm Hg suction was maintained in a circular area, 1 cm in diameter, on the outer side of the upper arm, for 1 minute, and the number of petechiae produced was counted.

Coagulation Time The method of Lee and White was employed.¹⁰ The tests were done at room temperature. Two cubic centimeters of blood were placed in each of 2 clean glass tubes 8 × 75 mm in diameter. The first tube was tipped gently at one-half minute intervals until it could be inverted without spilling. Then the second tube was tipped likewise until it could be inverted. The coagulation time was recorded as the interval between the injection of the blood into the first tube and the coagulation of the blood in the second tube.

RESULTS

A Normal Standards and Distribution of Results in the Normal and Abnormal Groups

Platelet Count The values for the normal platelet count were mean, 409,000 per cubic millimeter, standard deviation, 68,000 per cubic millimeter, normal range ($M \pm 2\sigma$), 273,000 to 545,000 per cubic millimeter. The distribution of the platelet counts done on 64 normal subjects and on 404 patients suffering from various diseases is given in figure 1.

Clot Retraction The normal values for the fluid volume of the clot were mean, 7.9 per cent, standard deviation, 6.0 per cent, normal range ($M \pm 2\sigma$) -4.1 per cent to 19.9 per cent.* The distribution of results of the clot retraction measure-

*Negative values may be due either to the error of the method or to tighter packing of the erythrocytes by the process of clot retraction than by centrifugalization at 3000 r.p.m. for 30 minutes.

ment done on 64 normal subjects and on 404 patients suffering from various diseases is given in figure 2

Bleeding Time The normal values for the bleeding time were mean, 3.2 min-

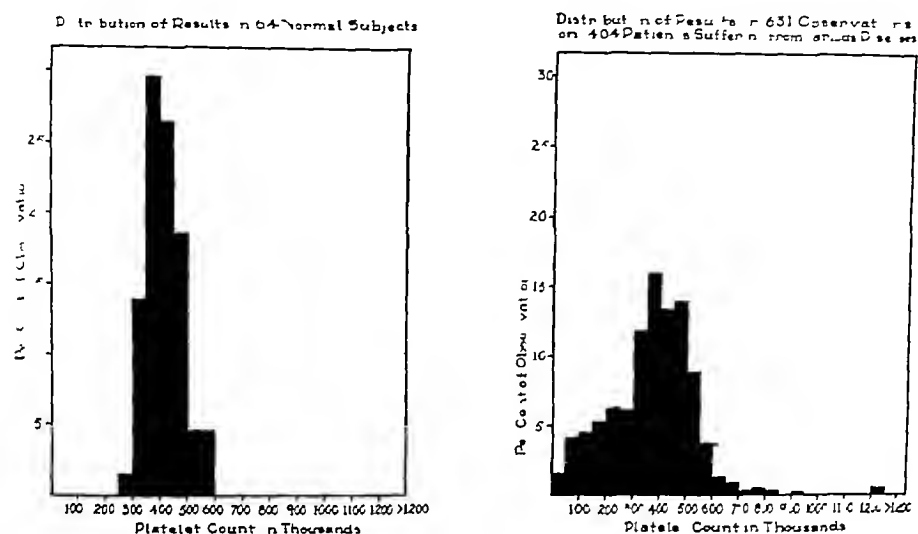


FIG. 1. RESULTS OF THE PLATELET COUNT—REES AND ECKER METHOD

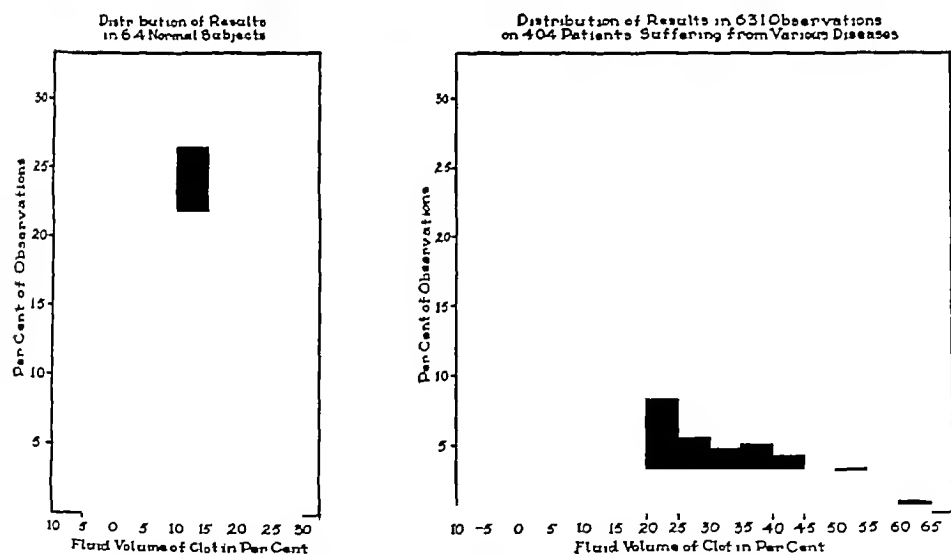


FIG. 2. RESULTS OF CLOT RETRACTION MEASUREMENT

utes, standard deviation, 1.6 minutes, normal range ($M \pm 2\sigma$), 0 to 6.4 minutes. The distribution of results of tests done on 64 normal subjects and on 404 patients suffering from various diseases is shown in figure 3.

Capillary Fragility There were 10 or less petechiae in 92 per cent of the normal

subjects studied, and this value was accepted as normal. The distribution of results of tests of the capillary fragility done on 64 normal subjects and on 404 patients suffering from various diseases is given in figure 4.

Coagulation Time The normal values for the coagulation time were mean, 8.9 minutes, standard deviation, 2.7 minutes, normal range ($M \pm 2\sigma$), 3.5 to 14.3.

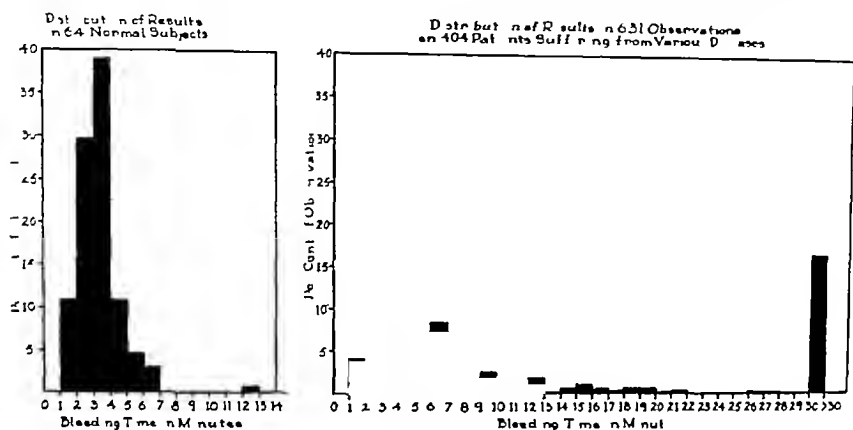


FIG. 3. RESULTS OF THE BLEEDING TIME TESTS—Ivy METHOD

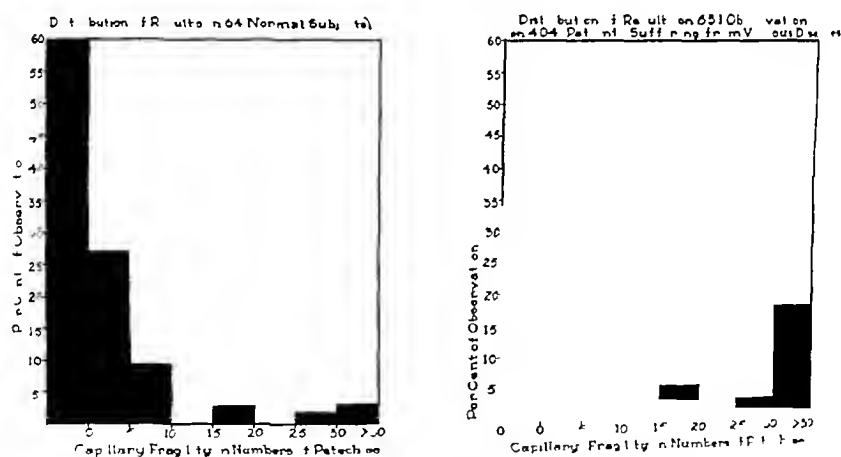


FIG. 4. RESULTS OF CAPILLARY FRAGILITY TEST—DALLORF METHOD

minutes. The distribution of results of the coagulation time tests done on 64 normal subjects and on 404 patients suffering from various diseases is given in figure 5.

B. Clinical Observations

Primary Thrombocytopenia The results of the initial tests made on 23 cases of primary thrombocytopenic purpura are given in table 3. Sixteen of the patients were females and 7 were males. Their ages varied between 2 and 79 years. The

duration of purpura was from a few days to 25 years. The patients remained under our direct care for periods varying from a few weeks to 4 years. Many were returned to other physicians after a short period of observation in our clinic, and although additional information on their course is available to us, in this study we have used only the data collected in our own laboratory.

The platelet counts during the active phases of purpura in all but 2 patients varied between 30,000 and 190,000 per cubic millimeter. Within this range there appeared to be no significant correlation between the level of the platelet count and the severity of the bleeding. In one patient (case 112, fig. 6) the platelet count varied between 30,000 and 120,000 per cubic millimeter over a period of 2 years. Her bleeding was of only moderate degree and was confined to the skin and oral mucous membranes. The platelet count eventually became normal and all symp-

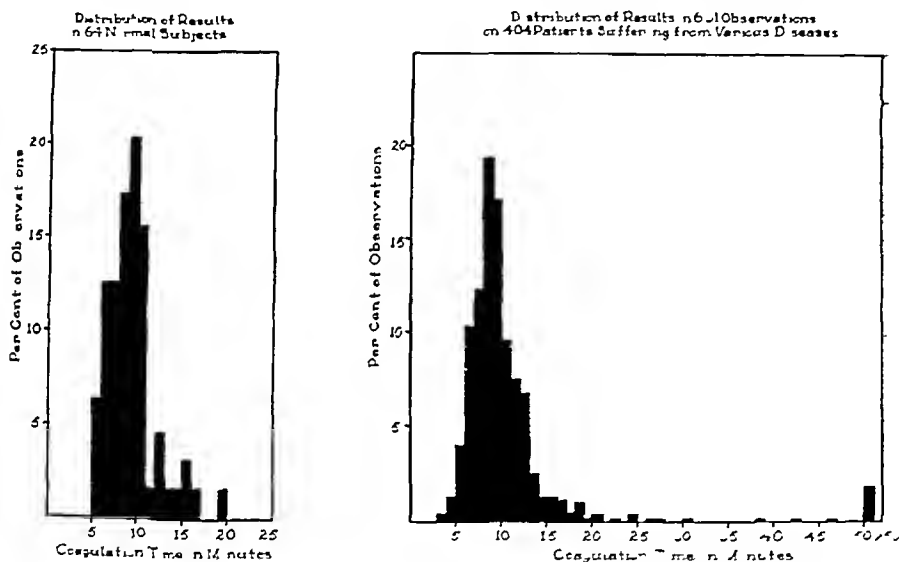


FIG. 5. RESULTS OF THE COAGULATION TIME TEST—LEE AND WHITE METHOD

toms disappeared. In contrast, another patient (case 176, fig. 8) had platelet counts varying between 70,000 and 250,000 per cubic millimeter over a period of 5 weeks. During this time he suffered from petechiae, ecchymoses, epistaxis, retinal hemorrhages, subpleural bleeding, gastrointestinal hemorrhage, bleeding into the right maxillary sinus, and bleeding into the tissues of the neck; he finally died of intracranial hemorrhage. We have recently studied another patient (case 575, fig. 8) who has suffered from petechiae and ecchymoses for the past 25 years. Twenty-five platelet counts done over a period of 5 months have varied between 180,000 and 280,000 per cubic millimeter with an average of approximately 220,000 per cubic millimeter. On three occasions, in which the platelet counts were 220,000, 260,000, and 280,000, the bleeding time was over 30 minutes, clot retraction was very poor, and capillary fragility was markedly increased.

The bleeding time was notably prolonged in every case in the series, in 21 of the 23 observations it was longer than 30 minutes. Clot retraction was markedly diminished in all cases the values for the fluid volume of the clot varied between 31 and 57 per cent. The capillary fragility was increased in 18 of 23 cases, however, a marked increase (over 30 petechiae) was present in only 15 cases. The prothrombin concentration was slightly reduced in 4 cases. In 2 patients there was a significant prolongation of the coagulation time. We believe that

TABLE 3 — *Primary Thrombocytopenic Purpura—Initial Observations*

Case number	Age in years	Sex	Coagulation time in minutes	Prothrombin concentration in per cent of normal	Platelet count per cu mm	Bleeding time in minutes	Fluid volume of clot in per cent	Capillary fragility in number of petechiae
40	40	F	7	75	70,000	>30	51	Shower*
112	79	F	7	90	170,000	>30	32	76
123	2	F	5	100	50,000	>30	49	0
132	12	F	10	80	70,000	>30	31	6
165	13	M	11½	60	120,000	>30	41	Shower
176	29	M	24	75	70,000	>30	51	Shower
186	20	M	14	90	170,000	>30	42	25
258	63	F	9½	100	100,000	>30	38	Shower
268	58	M	26½	70	190,000	>30	40	Shower
357	19	F	12½	65	190,000	>30	49	60
368	18	M	9½	80	50,000	15	39	51
467	1½	F	7	100	80,000	>30	45	5
484	18	F	8½	90	95,000	>30	52	Shower
537	28	F	7	90	80,000	19	39	Shower
542	24	F	8	65	110,000	>30	40	25
556	6	F	6	85	110,000	>30	40	Shower
557	17	F	9	100	50,000	>30	42	0
563	56	F	6½	100	30,000	>30	47	0
565	23	F	8½	100	140,000	>30	37	14
566	16	M	10	85	60,000	>30	32	Shower
567	39	M	6	100	90,000	>30	37	Shower
570	35	F	10	90	50,000	>30	57	41
575	57	F	14	90	260,000	>30	36	Shower

* Shower indicates that the petechiae were too numerous to count accurately

both of these instances were due to deficient platelet function. The case of 1 patient (176) has been reported in detail elsewhere.¹¹

The further course of the platelet count in many of the cases in this series is given in figures 6-9. In figures 6 and 7 are shown 4 instances of spontaneous recovery. In figure 8 the platelet counts in 4 patients who did not recover under conservative treatment are given. The platelet counts in 6 patients subjected to splenectomy are shown in figure 9.

Secondary Thrombocytopenia The results of the initial tests made on 28 cases of secondary thrombocytopenic purpura are given in table 4. Thirteen of the patients were females and 15 were males. Their ages varied between 8 and 65 years. The

duration of the bleeding tendency before coming under our observation was variable but was in general shorter than in primary thrombocytopenic purpura.

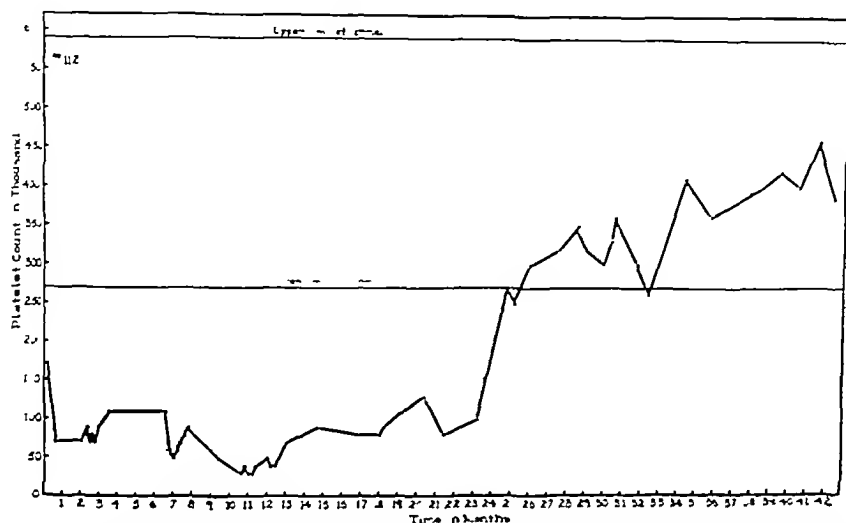


FIG 6 SPONTANEOUS RECOVERY FROM PRIMARY THROMBOCYTOPENIC PURPURA IN AN 81 YEAR OLD WOMAN
All symptoms disappeared when the platelet count returned to normal

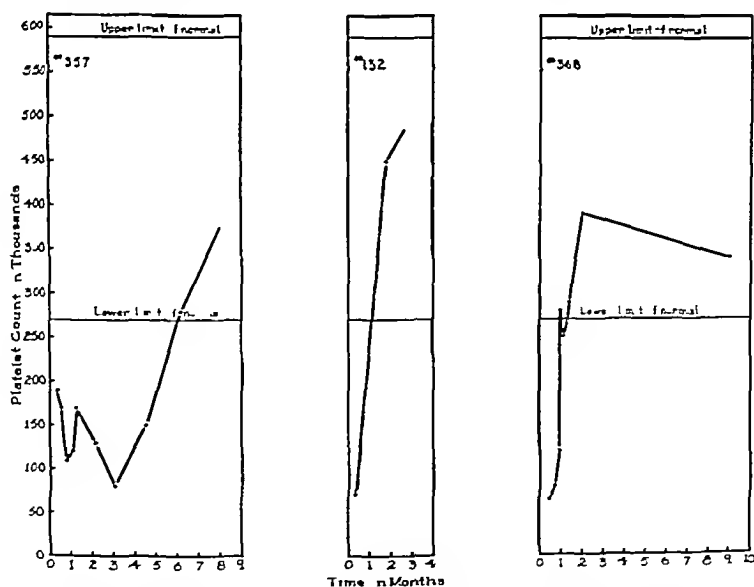


FIG 7 SPONTANEOUS RECOVERY FROM PRIMARY THROMBOCYTOPENIC PURPURA

In all patients the symptoms disappeared when the platelet count returned to normal

Some patients developed purpura while under treatment in our clinic for the associated diseases. Many of the patients died of the underlying disease while under

our care. Because of the nature of the conditions under which the data were collected, there is a preponderance of cases due to hematological disorders.

The initial platelet counts varied between 20,000 and 240,000 per cubic milli-

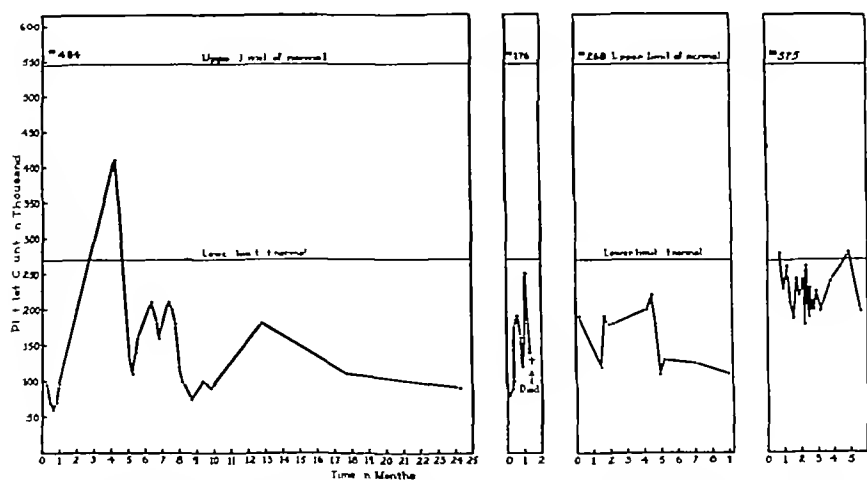


FIG 8 FAILURE TO RECOVER FROM PRIMARY THROMBOCYTOPENIC PURPURA UNDER CONSERVATIVE TREATMENT

Death in case 176 resulted from intracranial hemorrhage

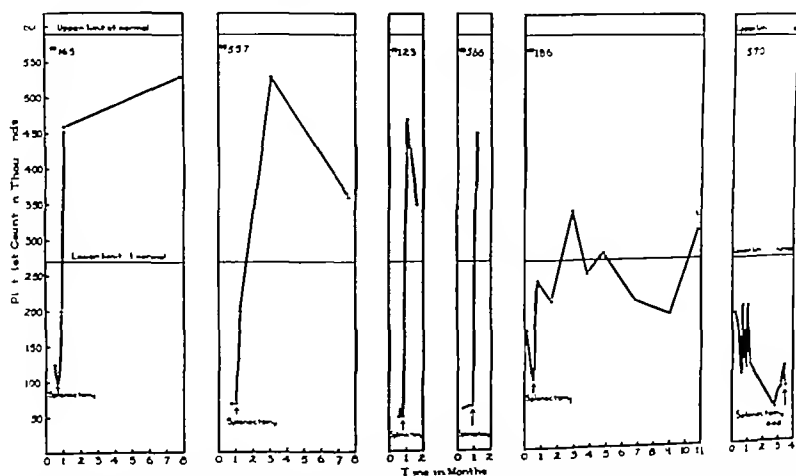


FIG 9 THE EFFECT OF SPLENECTOMY ON THE PLATELET COUNT IN PRIMARY THROMBOCYTOPENIC PURPURA

In all patients the symptoms disappeared immediately following the operation except in case 570, which terminated fatally 8 hours after splenectomy.

meter. The average of the platelet counts was 140,000 per cubic millimeter as compared with an average of approximately 100,000 per cubic millimeter in primary thrombocytopenic purpura.

The bleeding time was markedly prolonged in 18 cases, moderately prolonged in 7, slightly prolonged in 1, and normal in 2. Clot retraction was diminished in 25 cases and normal in 3. Capillary fragility was markedly increased in 11 cases, slightly or moderately increased in 4, and normal in 13. The prothrombin con-

TABLE 4—*Secondary Thrombocytopenic Purpura—Initial Observations*

Thrombocytopenia associated with	Case number	Age in years	Sex	Coagulation time in minutes	Prothrombin concentration in per cent	Platelet count per cu mm	Bleeding time in minutes	Fluid volume of clot in per cent	Capillary fragility in numbers of petechiae
Refractory Anemia	63	34	F	8½	75	130,000	>30	55	16
Refractory Anemia	153	49	M	11	60	100,000	>30	42	2
Refractory Anemia	219	29	F	10	76	20,000	>30	60	Shower*
Refractory Anemia	251	38	F	11½	70	130,000	>30	53	38
Refractory Anemia	367	36	F	12½	90	190,000	>30	54	Shower
Refractory Anemia	518	44	M	9	75	110,000	>30	56	10
Refractory Anemia	526	65	M	9	60	30,000	>30	49	37
Refractory Anemia	50	42	M	9	55	160,000	27	50	Shower
Pernicious Anemia	183	56	F	11½	60	230,000	>30	41	0
Myelophthisic Anemia	216	63	M	9½	35	210,000	14	24	0
Multiple Myeloma	274	52	M	20	95	190,000	12	43	15
Infectious Mononucleosis	225	15	M	10½	75	90,000	>30	54	28
Banti's Syndrome	93	8	F	7½	90	60,000	8	37	30
Acute Lymphatic Leukemia	52	25	F	6½	60	190,000	>30	37	0
Acute Lymphatic Leukemia	175	16	M	7	65	70,000	>30	46	5
Acute Lymphatic Leukemia	275	16	M	3½	65	200,000	>30	39	0
Acute Lymphatic Leukemia	490	20	M	6	100	70,000	10	9	1
Subacute Lymphatic Leukemia	476	31	F	8½	100	150,000	>30	33	32
Chronic Lymphatic Leukemia	3	60	M	5	75	40,000	11	41	7
Chronic Myelogenous Leukemia	11	22	F	9½	50	210,000	2	8	0
Chronic Myelogenous Leukemia	334	28	M	8	50	230,000	>30	6	1
Chronic Myelogenous Leukemia	460	61	M	16	80	230,000	12	55	5
Monocytic Leukemia	212	28	F	6	50	60,000	>30	59	Shower
Monocytic Leukemia	396	63	M	8	90	240,000	>30	36	12
X ray Therapy	149	45	F	9	75	90,000	17	27	Shower
X ray Therapy	248	31	F	8	80	230,000	>30	53	94
Streptococcus Sore Throat	265	12	M	7	75	170,000	6	27	10
Bismarsen Sensitivity	428	10	F	10	75	120,000	12	27	Shower

* Shower indicates that the petechiae were too numerous to count accurately.

centration was diminished in 12 cases and normal in 16. The coagulation time was prolonged in 2 cases and normal in 16.

In figure 10 are shown the platelet counts in a patient suffering from chronic myelogenous leukemia with terminal thrombocytopenic purpura, and the counts in a patient who developed thrombocytopenic purpura in the course of infectious mononucleosis and gradually recovered as the underlying disease subsided. In

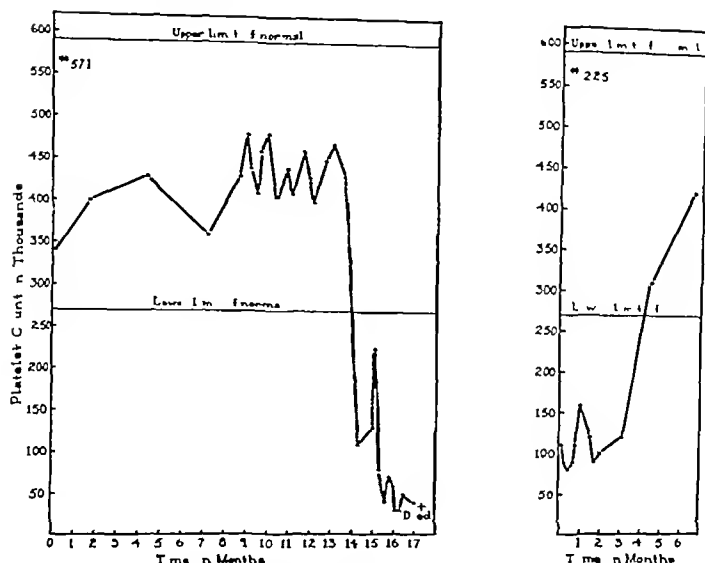


FIG 10

TERMINAL THROMBOCYTOPENIC PURPURA IN A PATIENT SUFFERING FROM CHRONIC MYELOGENOUS LEUKEMIA

SPONTANEOUS RECOVERY FROM THROMBOCYTOPENIC PURPURA ASSOCIATED WITH INFECTIOUS MONONUCLEOSIS

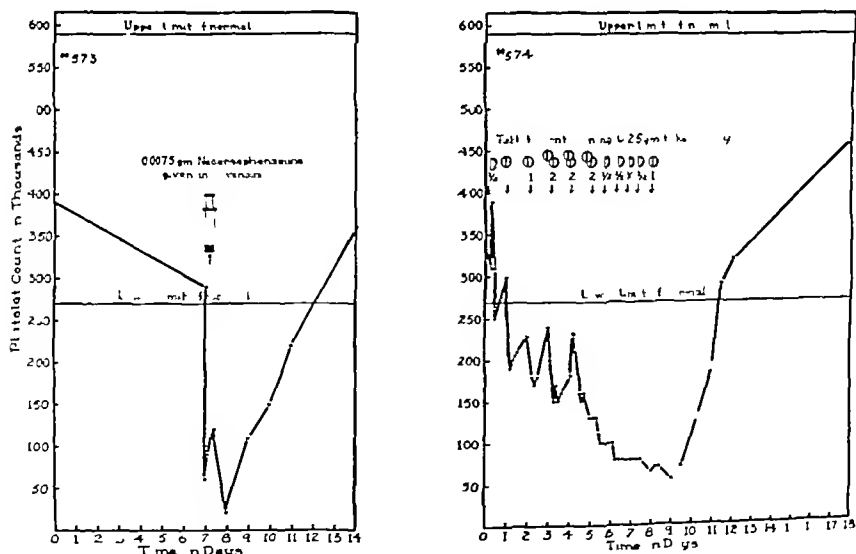


FIG 11

SECONDARY THROMBOCYTOPENIC PURPURA INDUCED IN A SENSITIVE PATIENT BY THE ADMINISTRATION OF NEOARSEPHEMINAMINE

SECONDARY THROMBOCYTOPENIC PURPURA INDUCED IN A SENSITIVE PATIENT BY THE ADMINISTRATION OF SEDORMID

figures 11 and 12 are shown the platelet counts in 3 patients after the administration of drugs to which they were known to be sensitive * Purpura developed in all patients when the platelet count was reduced and disappeared with the return of the platelet count to the normal range

Thrombocytopenia without Bleeding The results of the tests made on 19 cases of secondary thrombocytopenia without bleeding are given in table 5 As would be expected, the level of the platelet counts was in general higher than in patients with purpuric symptoms The average of the platelet counts was 210,000 per cu mm as compared with 100,000 per cu mm in primary thrombocytopenic purpura and 140,000 per cu mm in secondary thrombocytopenic purpura The bleeding time was markedly prolonged in 2 cases, moderately prolonged in 5,

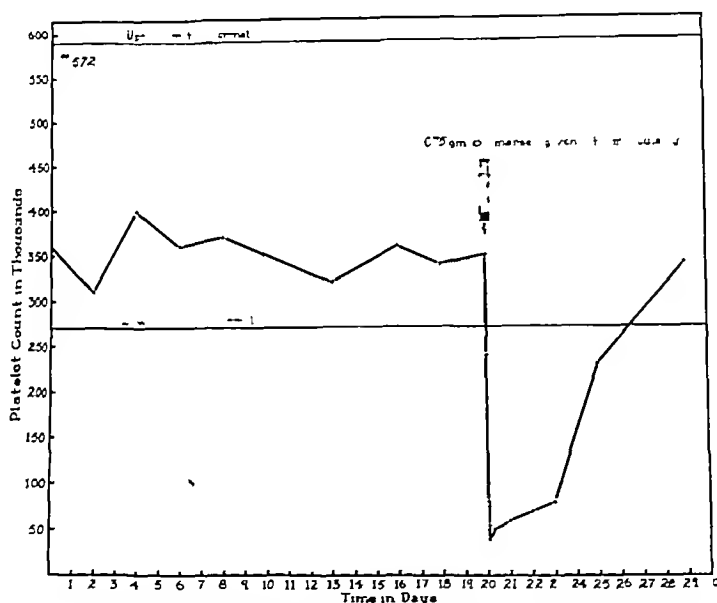


FIG 12 SECONDARY THROMBOCYTOPENIC PURPURA INDUCED IN A SENSITIVE PERSON BY THE ADMINISTRATION OF BISMARSEN

slightly prolonged in 2, and normal in 10 Clot retraction was diminished in 8 cases and normal in 11 Capillary fragility was markedly increased in 6 cases, slightly increased in 2, and normal in 11 The prothrombin concentration was diminished in 4 cases and normal in 15 The coagulation time was normal in all cases

Further studies on a patient with refractory anemia who suffered from thrombocytopenia without abnormal bleeding are shown in figure 13 It is interesting to note that during the period of rapid fluctuations in her platelet count, the

*These cases have been reported in detail by Dr Ernest Falconer,^{12,13} who has kindly granted us permission to use his data The platelet counts were done by the same technician responsible for the remainder of the platelet counts in the present study

TABLE 5—*Secondary Thrombocytopenia without Bleeding*

Thrombocytopenia associated with	Case number	Age in years	Sex	Coagulation time in minutes	Prothrombin concentration in per cent	Platelet count per cu. mm	Bleeding time in minutes	Fluid volume of clot in per cent	Capillary fragility in numbers of petechiae
Chronic Lymphatic Leukemia	1264	M	9	95	140,000	4	23	5	
Chronic Lymphatic Leukemia	24967	M	9	100	260,000	5	16	32	
Chronic Lymphatic Leukemia	36159	M	7	100	260,000	12½	52	0	
Chronic Myelogenous Leukemia	54920	M	10	95	210,000	2½	5	0	
Lymphosarcomatosis	5850	M	8½	90	130,000	16½	14	11	
Reticuloendotheliosis	654½	M	9	75	200,000	14	8	64	
Refractory Anemia	23148	F	13	85	210,000	10	25	35	
Refractory Anemia	49317	F	9	100	260,000	12½	52	12	
Refractory Anemia	16922	F	10½	100	100,000	24½	26	Shower	
Multiple Myeloma	10443	M	10	75	210,000	>30	31	3	
Banti's Syndrome	12220	F	8½	35	190,000	9½	26	1	
Primary Carcinoma of Liver	38638	M	8	50	190,000	7	36	Shower	
Portal Cirrhosis of Liver	17728	M	12	40	160,000	3½	10	5	
Obstructive Jaundice	37261	M	6	75	230,000	1½	13	0	
Diabetes Mellitus	989	F	8	60	260,000	5	14	0	
Toxic Erythema	230½	F	9	100	250,000	2½	2	0	
Psychoneurosis	43336	F	9½	100	210,000	4	6	4	
Anorexia Nervosa	32820	F	11	95	230,000	6	11	1	
Internal Hemorrhoids	42263	M	9	75	210,000	2	9	36	

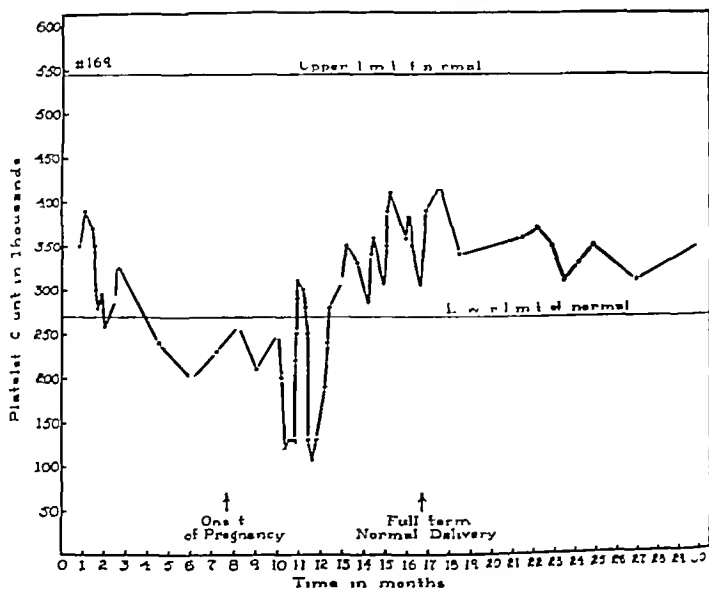


FIG 13 THROMBOCYTOPENIA WITHOUT PURPURA IN A PATIENT SUFFERING FROM REFRACTORY ANEMIA

bleeding time was markedly prolonged, clot retraction was diminished, and capillary fragility was increased, regardless of the level of the platelet count

Thrombocytopenia Complicating Other Hemorrhagic States The results of tests in 15 cases of thrombocytopenia complicating other hemorrhagic conditions are given in table 6. In many instances the segregation of these cases from the group of patients classified as having secondary thrombocytopenic purpura was difficult. Cases were included in this category if the further course of the patient's illness showed that thrombocytopenia was not the principal cause of the bleeding or if the thrombocytopenia was found to be inconstant despite persistence of symptoms of abnormal bleeding. The average of the platelet counts in this group (225,000 per cu mm) was slightly higher than that found in secondary thrombocytopenia

TABLE 6—*Thrombocytopenia Complicating Other Hemorrhagic States*

Thrombocytopenia associated with	Case number	Age in years	Sex	Coagulation time in minutes	Prothrombin concentration in per cent	Platelet count per cu mm	Bleeding time in minutes	Final volume of clot in per cent	Capillary fragility in numbers of petechiae
Hemophilia	51	25	M	300	100	190,000	4½	11	16
Hemophilia	399	1½	M	46	70	250,000	> 30	23	0
Von Willebrand's Syndrome	425	53	F	9	100	220,000	9	6	Shower
Von Willebrand's Syndrome	517	6	M	9½	75	190,000	> 30	26	6
Arterial Hypertension	15	47	F	6½	100	200,000	13½	0	0
Arterial Hypertension	33	5-	M	8½	100	240,000	> 30	18	Shower
Chronic Pyelonephritis	373	18	M	12	100	250,000	11½	4	1
Atypical Purpura	96	64	M	9½	90	240,000	6	11	33
Atypical Purpura	214	33	F	12½	65	260,000	6½	47	9
Portal Cirrhosis of Liver	27	53	M	6½	25	240,000	8½	54	14
Portal Cirrhosis of Liver	178	55	M	6	30	180,000	4½	34	0
Portal Cirrhosis of Liver	329	42	F	8	45	200,000	6½	52	0
Portal Cirrhosis of Liver	482	48	F	4	35	230,000	6	19	3
Subacute Yellow Atrophy of Liver	453	38	M	9½	25	230,000	4	15	0
Obstructive Jaundice	107	65	F	8½	40	260,000	5½	24	15

without bleeding. The bleeding time was markedly prolonged in 3 cases, moderately prolonged in 2, slightly prolonged in 2, and normal in 8. Clot retraction was diminished in 7 cases and normal in 8. Capillary fragility was increased in 6 cases and normal in 9. The prothrombin concentration was reduced in 8 cases and normal in 7. The coagulation time was markedly prolonged in 2 patients, both of whom suffered from hemophilia.

Further studies on a patient with an hereditary hemorrhagic syndrome who suffered from intermittent periods of thrombocytopenia are shown in figure 14. Twenty-six series of tests were done on this patient over a period of 2 years. On every occasion, regardless of the level of the platelet count, the bleeding time was markedly prolonged and the capillary fragility was increased. Clot retraction was

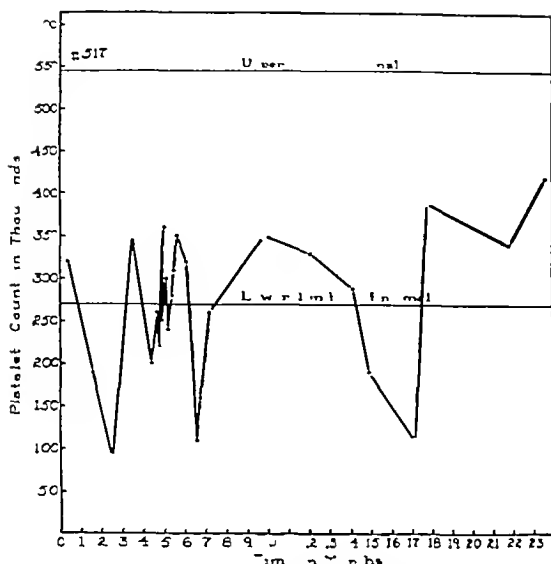


FIG. 14. RECURRENT THROMBOCYTOPENIA IN A PATIENT SUFFERING FROM AN HEREDITARY HEMORRHAGIC SYNDROME CHARACTERIZED BY INCREASED CAPILLARY FRAGILITY AND MARKEDLY PROLONGED BLEEDING TIME (VON WILLEBRAND TYPE)

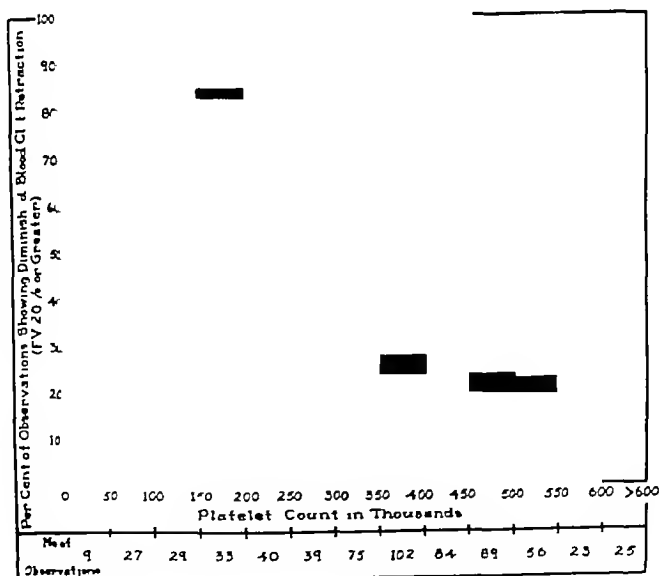


FIG. 15. THE ASSOCIATION OF DIMINISHED CLOT RETRACTION WITH THE PLATELET COUNT

quite variable, it was frequently decreased but appeared to bear no significant relationship to the level of the platelet count, it was sometimes diminished in the presence of a normal platelet count and was sometimes normal in the presence of marked thrombocytopenia

C Statistical Relationships

Platelets and Clot Retraction The coefficient of correlation between the platelet counts and the results of the clot retraction measurement was significant (-0.55

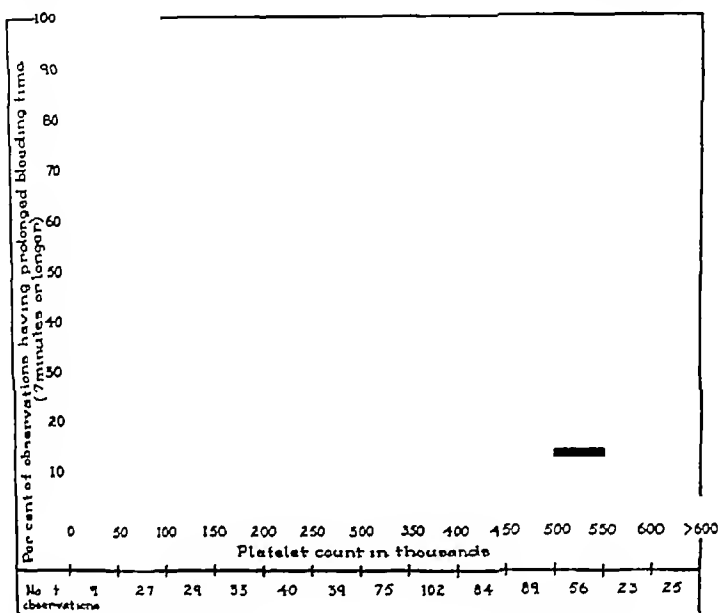


FIG 16 THE ASSOCIATION OF PROLONGED BLEEDING TIME WITH THE PLATELET COUNT

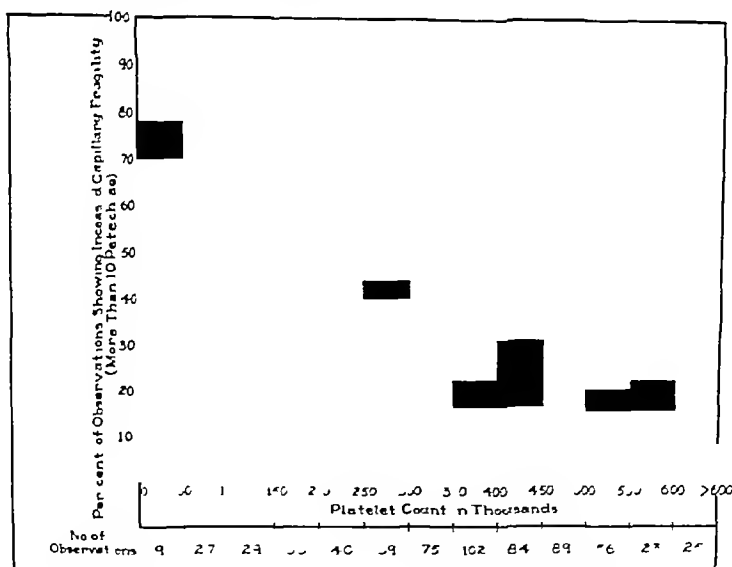


FIG 17 THE ASSOCIATION OF INCREASED CAPILLARY FRAGILITY WITH THE PLATELET COUNT

± 0.03), and the coefficient of association (Yule's Q) between decreased platelet counts and diminished clot retraction (0.88 ± 0.01) was also significant. The

association of diminished clot retraction with the platelet count is shown in figure 15

Platelets and Bleeding Time The coefficient of correlation between the platelet count and the bleeding time was significant (-0.59 ± 0.02), and the coefficient of association (Yule's Q) between decreased platelet counts and prolonged bleeding time was also significant (0.87 ± 0.03). The association of prolonged bleeding time with the platelet count is shown in figure 16

Platelets and Capillary Fragility The coefficient of correlation between the platelet count and the results of the capillary fragility test was significant (-0.38 ± 0.03), and the coefficient of association (Yule's Q) between decreased platelet counts and increased capillary fragility was also significant (0.70 ± 0.05). The

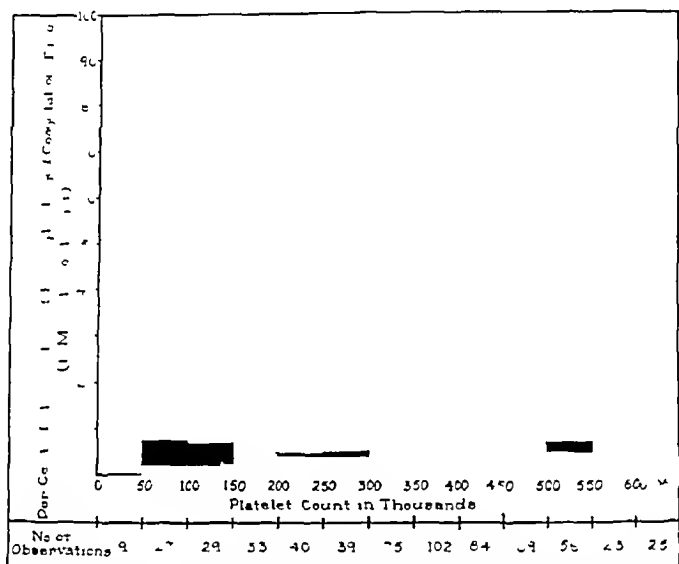


FIG. 18 THE ASSOCIATION OF PROLONGED COAGULATION TIME WITH THE PLATELET COUNT

association of increased capillary fragility with the platelet count is shown in figure 17

Platelets and Coagulation Time There was no significant correlation between the platelet count and the coagulation time and no significant association of thrombocytopenia with prolonged coagulation time. The coefficient of correlation was -0.21 ± 0.04 , and the coefficient of association (Yule's Q) 0.20 ± 0.16 . The association of prolonged coagulation time with the platelet count is shown in figure 18

DISCUSSION

The Normal Range and Critical Level of the Platelet Count

In any bleeding tendency, the unequivocal demonstration of a reduction in the platelet count is significant. This does not preclude the possibility that other

factors might contribute to the bleeding tendency, nor does it imply that all instances of thrombocytopenia are accompanied by abnormal bleeding. However, when the platelet count is reduced, there is at least a theoretical possibility that the patient will bleed more readily than would a patient with a normal platelet count. Even slight reductions in the platelet count are therefore of prognostic significance.

Although it has been emphasized that the normal platelet count in man may vary between 100,000 and 1,000,000 per cubic millimeter, depending upon the methods employed,¹⁴ the normal range is usually given as 200,000 to 400,000 per cu mm. Furthermore, it is not generally appreciated that there may be wide variations in the results obtained by different observers, each using the same method of counting. The individual differences appear to depend upon the microscopic technic and visual acuity of the observer. We are convinced that it is impossible to standardize the platelet count so that the results of even expert technicians will not show significant variation. We are equally certain, however, that with practice and constant attention to technical details the results of a single technician may attain a high degree of reliability. Because of these individual variations, it is impossible to state the normal range of the platelet count for any given method in values that would be universally acceptable. Values which would be well within the normal range when obtained by one technician might be grossly abnormal if found by another. The only solution to this problem appears to be for each observer to establish his own standards of normality. In our laboratory during the period of this study the normal range was 273,000 to 545,000 per cu mm.

There have been few statistical analyses of the normal platelet count reported in the literature. It is interesting to compare our values with those given by Tocantins,¹⁵ whose figures for cutaneous blood on 40 young adult white male subjects were mean, 250,000 per cu mm, probable error of mean, 7,458 per cu mm, standard deviation, 58,500 per cu mm. The normal range ($M \pm 2\sigma$) using these values would be 133,000 per cu mm to 367,000 per cu mm. It is obvious that platelet counts which would be grossly abnormal by our standards might be well within the normal range found by Tocantins. Yet the reliability of our values as compared with those of Tocantins, using the same statistical methods of analysis, is almost identical. These results furnish abundant proof of the necessity for individual standardization of the platelet count.

If it is difficult to give a universally acceptable normal range, it is equally difficult to define the critical level of the platelet count below which the patient is likely to manifest symptoms of purpura. It is obviously not a fixed value such as 50,000 to 75,000 per cubic millimeter, as is so often stated in the literature. In our laboratory the critical level of the platelet count was found to be approximately 190,000 per cu mm in primary thrombocytopenic purpura and 230,000 per cu mm in secondary thrombocytopenic purpura. With platelet counts between 230,000 and 270,000 per cu mm, abnormal bleeding was usually either not present or was associated with some cause other than the reduced platelet count. However, in a very few instances classical thrombocytopenic purpura was observed

within this range of platelet counts. The term "critical level" of the platelet count should therefore be used only to signify the usual level at which symptoms of purpura are likely to appear. It should not imply that thrombocytopenic bleeding may not occur with platelet counts above the critical level.

Clot Retraction

The mechanism whereby the platelets affect clot retraction has been carefully studied by Tocantins.¹⁶ After coagulation of the blood has occurred, intact platelets migrate toward the fibrin strands and become adherent to them. These masses of clumped platelets form nuclei from which new needles of fibrin radiate. Within a short time many of the platelet groups fuse and thus cause the fibrin strands to become twisted, bent, and shortened. It is in this manner that the fluid is expressed from the clot, making it a firm, dense, elastic body.

The most frequently observed cause of diminished clot retraction, other than a low platelet count, appears to be a lowered prothrombin concentration. Boyce and McFetridge^{17, 18} noted diminished clot retraction in patients suffering from obstructive jaundice, and Aggeler and Lucia¹⁹ found a correlation coefficient of 0.450 ± 0.09 in 85 paired determinations of the clot retraction and the prothrombin concentration on a group composed of 3 normal subjects and 41 patients suffering from obstructive jaundice or diseases affecting the liver. In a more recent analysis of a larger series of determinations* we have found no significant correlation of clot retraction and prothrombin concentration, nor any significant association of diminished clot retraction with hypoprothrombinemia, either in a group of patients suffering from various diseases, or when the group studied was restricted to patients suffering from obstructive jaundice or diseases affecting the liver. It was only in the determination of the *correlation ratio* in a group restricted to patients suffering from obstructive jaundice that a significant relationship was found. Since many of our patients suffered from obstructive jaundice, it is probable that some of the observations of diminished clot retraction in our series are associated with hypoprothrombinemia and are not related to platelet function.

Another factor, seldom appreciated, which may influence quantitative measurements of clot retraction, is the relationship between the coagulation time of the blood and the suspension stability of the erythrocytes. When the coagulation time is markedly prolonged, as in hemophilia, or the sedimentation rate is extremely rapid, as in multiple myeloma, the erythrocytes may settle to the bottom of the test tube before the blood has coagulated, producing a buffy coat clot. Because the upper part of such a clot is relatively free of erythrocytes, the space between the interlacing strands of fibrin is occupied principally by occluded fluid. We have repeatedly demonstrated that the fluid occluded within the clot of patients suffering from hemophilia can be markedly reduced by transfusion of whole blood or plasma. The resultant shortening of the coagulation time results in disappearance of the buffy coat with its relatively large pool of fluid. It is probable therefore that some instances of diminished clot retraction in our series are due either to delayed coagulation of the blood or to very rapid sedimentation of the

*Unpublished data

erythrocytes, or to both. It is even possible that the significant correlation ratio found between the prothrombin concentration and clot retraction in obstructive jaundice may be due to these factors.

The effect of a deficient concentration of fibrinogen on clot retraction appears to be of little practical importance since so few instances of this abnormality are observed in clinical practice. When the fibrinogen is markedly diminished there will be formed only a few strands of fibrin which are incapable of binding the cellular elements of the blood into a clot. It is possible, however, that slight or moderate reductions in the fibrinogen concentration might result in the formation of a clot which would be defective in its ability to retract. We have not studied this specific problem. In every patient in our series there was a sufficient amount of fibrin formed to produce a definite coagulum. Fibrinogen determinations were done on approximately 100 patients, and although many high values were recorded, none were abnormally low. However, since fibrinogen determinations were not done on all patients, it is possible that some instances of diminished clot retraction were due to deficiencies in this factor. The converse appears not to be true, there was no significant association of high fibrinogen concentration with normal clot retraction.

An *increased* degree of clot retraction appears to be an impossibility since a normal clot may be totally devoid of occluded fluid. It is interesting to note, however, that diminished clot retraction occurred only once in 48 observations in which the platelet count was higher than the upper limit of its normal range. This finding suggests that if enough platelets are present they may overcome practically all influences tending to produce diminished clot retraction.

Increases in the *rate* should be distinguished from increases in the *degree* of clot retraction. Hirschboeck²⁰ has devised a method for detecting an increased rate of retraction which is stated to be of value in detecting the probability of thrombophlebitis or pulmonary infarct. Tocantins' test²¹ is also, to some degree, dependent upon the speed of retraction of the clot.

Aggeler and Lucia²² have recently disproved the contention of Rabinowitz²³ that clot retraction may be influenced by various amino acids.

Bleeding Time

The mechanism whereby the platelets influence the bleeding time is obscure. The immediate cessation of bleeding is caused by retraction of the capillaries in response to injury and is not due to thrombus formation, coagulation of the blood, or retraction of the blood clot. Macfarlane²⁴ believes that failure of the capillaries to retract in thrombocytopenic purpura may be a coincidental abnormality, not causally related to the platelet count.

In the absence of thrombocytopenia, the principal conditions in which a prolonged bleeding time was found were familial purpura (Von Willebrand type), obstructive jaundice, diseases affecting the liver, malignant hypertension, diseases affecting the kidneys, subacute bacterial endocarditis, and disseminated lupus erythematosus. It is probable that in many of these conditions the prolonged bleeding time was due to vascular abnormalities not related to platelet function. In a

few instances the prolonged bleeding time associated with a low prothrombin concentration returned to normal when the prothrombin concentration was elevated following the administration of vitamin K. Since in most of the instances in which this occurred there was no other change in the status of the patient, one must conclude that defects in the blood coagulation mechanism can affect the bleeding time. However, this is probably true only if other purpurogenic factors, not sufficient in themselves to prolong the bleeding time, are also present. We have likewise occasionally observed a prolonged bleeding time in hemophilia, and in all instances in which this occurred there was ample evidence of a concomitant vascular abnormality. The majority of the determinations of the bleeding time in untreated hemophilia were normal.

Capillary Fragility

The mechanism whereby the platelets participate in maintaining capillary continuity is unknown. It has been suggested that the platelets may protect the capillaries by accumulating along weakened spots in the endothelium, thereby acting as a seal, hindering the escape of red blood cells. Quick²⁵ believes that they may function by removing from the blood a histamine like compound which in their absence would accumulate and cause undue vascular dilatation.

The most frequent cause of increased capillary fragility, other than thrombocytopenia, is marked vitamin C deficiency. The plasma vitamin C level was tested in a large number of the patients in our series. Many were found to have low values. In some the capillary resistance increased after treatment with vitamin C. Some instances of increased capillary fragility in our series can therefore be attributed to vitamin C deficiency and are not related to deficient platelet function.

Szent-Gyorgi²⁶ has recently described another factor, vitamin P, which is reputed to be superior to vitamin C in the prevention of bleeding due to increased capillary fragility. However, since its vitamin nature has not been established, one cannot state that vitamin P deficiency is a cause of increased capillary fragility. No investigations regarding vitamin P were conducted in our patients.

It is probable that many instances of increased capillary fragility in our series were due to the presence of vasculo-toxic substances. The results of the tests may also have been influenced by the thickness, color, and texture of the skin.

Blood Coagulation

In shed blood some of the platelets break up to supply thromboplastin, which is necessary for coagulation of the blood. Only a few normal platelets are required to fulfill this function. Hence the coagulation time is usually normal in the presence of thrombocytopenia but may be prolonged if the platelets are defective.

Despite the absence of a significant statistical relationship between the coagulation time and the platelet count there are instances reported of prolongation of the coagulation time in thrombocytopenic purpura. We have recently observed a case in which the coagulation time was found to be as long as 175 minutes.¹¹ All other causes of delayed coagulation of the blood were excluded by appropriate tests, and we concluded that the prolonged coagulation time in this case was due to

deficient platelet function. It is probable that other instances of prolonged coagulation time associated with thrombocytopenia in our series may be due to deficient platelet function. Whether the coagulation defect in these cases is identical with that found in hemophilia is problematical. The most commonly accepted hypothesis of the pathogenesis of hemophilia is that it is due to thromboplastin deficiency caused by a decreased rate of lysis of the platelets. On the other hand, the group working at the Thorndike Memorial Laboratory²⁷⁻³⁰ believe that the defect in coagulation is due to the absence of a substance which is normally present in the globulin fraction of blood.

Delayed blood coagulation may be caused by deficiencies of any of the elements concerned in coagulation or by the presence of anticoagulant substances. Many of the prolonged coagulation times in our series were associated with very low prothrombin concentrations. Although calcium and fibrinogen concentrations were tested only where they seemed indicated, we feel confident that in no case was a reduction of these factors responsible for delayed coagulation of the blood. There were several instances of prolonged coagulation time in patients who suffered from uremia, and it is possible that they were due to the retention of anticoagulant substances by the diseased kidneys. Patients who had been given anticoagulants (dicumarol or heparin) were not included in this series.

Laboratory Tests in the Diagnosis of Thrombocytopenic Purpura

In all cases of primary thrombocytopenic purpura observed in our clinic during the active phases of bleeding, clot retraction was definitely diminished and the bleeding time was markedly prolonged. In approximately three fourths of the cases capillary fragility was increased. However, in the stage of recovery, either spontaneous or induced by splenectomy, the results of one or all of these tests were sometimes found to be normal before the platelet count had returned to the normal range.

In a few patients suffering from secondary thrombocytopenic purpura during the active phase of bleeding, the tests of the bleeding time or clot retraction were normal. Only half of the patients in this group exhibited increased capillary fragility.

In the group of patients who suffered from secondary thrombocytopenia without abnormal bleeding, or from thrombocytopenia complicating other hemorrhagic states, in less than half of the patients the tests of the bleeding time, clot retraction, and capillary fragility were abnormal, and in general the degree of abnormality was less than that observed in primary or secondary thrombocytopenic purpura.

Our data suggest that when hemorrhagic symptoms are associated with either a normal bleeding time or normal clot retraction, they are not due to primary thrombocytopenic purpura unless recovery is imminent. The converse is not true, the presence of marked abnormalities in the bleeding time, clot retraction, and capillary fragility does not exclude a diagnosis of secondary thrombocytopenic purpura. The implications of these findings with regard to splenectomy are obvious: if all of the corollary tests are abnormal, splenectomy should be seriously considered, if the bleeding time or clot retraction is normal the possibility of spontaneous re-

covery should be considered likely and splenectomy should probably be withheld. These observations are predicated on the use of the same laboratory techniques as were employed in this study.

Thrombasthenic Purpuras

There is a group of nonthrombocytopenic hemorrhagic syndromes characterized either by prolonged bleeding time, diminished clot retraction, increased capillary fragility, prolonged coagulation time, or a combination of these abnormalities.²¹ It has been suggested that some of these conditions may be due to deficient platelet function or thrombasthenia. No convincing experimental evidence has been presented to prove this contention. A diminution in the function of the total mass of platelets, because of their decreased numbers, is frequently associated with prolonged bleeding time, diminished clot retraction, and increased capillary fragility. In the majority of patients in the active phases of primary or secondary thrombocytopenic purpura all three of these abnormalities are present. In the stage of recovery from these diseases, some patients may be observed with normal platelet counts but with persisting abnormalities in one or all of the above mentioned tests. Such a condition might be termed thrombasthenia. However, this state is seldom accompanied by any purpuric symptoms and is usually followed within a short time by complete recovery. We have observed one patient suffering from an hereditary hemorrhagic state characterized by a markedly prolonged bleeding time and increased capillary fragility (Von Willebrand's syndrome) who had recurrent periods of thrombocytopenia. However, his hemorrhagic symptoms were of approximately the same intensity regardless of the level of the platelet count. Clot retraction measurements were variable. It is difficult to state whether the prolonged bleeding time and increased capillary fragility in this patient were due to thrombasthenia or to an independent vascular abnormality. The recurrent periods of thrombocytopenia would suggest a fundamental platelet defect.

During the entire period of this study we have observed no patients suffering from a hemorrhagic syndrome characterized solely by diminished clot retraction (Glanzmann's syndrome).

The statistical data presented in this paper neither prove nor disprove the concept of thrombasthenia. Rather, they suggest that there is some variation in the functional capacity of platelets in different individuals and in the same individual at different times but that this variation is probably confined within relatively narrow limits.

SUMMARY

Determinations of the platelet count, clot retraction, bleeding time, capillary fragility, and coagulation time were made in 64 normal subjects and in 404 patients suffering from various diseases.

The normal values for the platelet count done by an experienced technician using the Rees and Ecker method were mean, 409,000 per cu mm, standard deviation, 68,000 per cu mm, normal range ($M \pm 2\sigma$), 273,000 per cu mm to 545,000 per cu mm.

There was a statistically significant relationship between the platelet count and the results of tests of clot retraction, bleeding time, and capillary fragility, but there was no significant relationship between the platelet count and the coagulation time. Factors other than platelet count or platelet function which may influence the results of these tests are discussed.

The critical level of the platelet count, below which abnormal bleeding is likely to occur, was found to be approximately 190,000 per cu mm in primary thrombocytopenic purpura and 230,000 per cu mm in secondary thrombocytopenic purpura. However, platelet counts as low as 100,000 per cu mm were found in one patient without abnormal bleeding, and counts as high as 280,000 per cu mm were found in another patient with classical primary thrombocytopenic purpura.

In all patients in the active phase of bleeding in primary thrombocytopenic purpura and in most with secondary thrombocytopenic purpura, the bleeding time was markedly prolonged and clot retraction was definitely diminished. In approximately one half of the patients suffering from thrombocytopenia without bleeding or from thrombocytopenia complicating other hemorrhagic states, the results of these tests were abnormal. Capillary fragility was increased in approximately three fourths of the patients with primary thrombocytopenic purpura, one half with secondary thrombocytopenic purpura, and less than one half with thrombocytopenia without bleeding or with thrombocytopenia complicating other hemorrhagic states.

In the stage of recovery from thrombocytopenic purpura, dissociation of the results of the various tests was sometimes found. In some patients the platelet counts returned to normal but abnormalities persisted in the tests of the bleeding time, clot retraction, or capillary fragility. In other patients the results of one or all of these tests returned to normal before the platelet count had reached the normal range. These results have been interpreted as evidence of variability in the functional capacity of the platelets.

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PSEUDOHEMOPHILIA

CASE STUDY*

B. WOODBURY PERKINS, LIEUT. , M. C. , U. S. N. R.

INTRODUCTION

A CASE of a hereditary hemorrhagic disease has been studied and is recorded primarily because the family history offers an unusually good opportunity to trace the genealogical background in this particular instance. The only persistent abnormality found has been prolongation of the bleeding time. Such a condition, which is often hereditary and is characterized chiefly by a prolonged bleeding time in the presence of normal platelets, has been previously described and referred to as pseudohemophilia,^{1,2} or hereditary hemorrhagic thrombasthenia.^{3,4}

REPORT OF CASE

The patient, a 20 year old white American born male, was admitted to the hospital with a swollen, painful left knee, which had been twisted 9 days previously in a baseball game. Almost immediate swelling followed the trauma. He was treated conservatively by the family physician until 3 days prior to admission, when the joint was aspirated with removal of clear, straw colored fluid. Because of recurrence of joint pain 3 days following this tap the patient was hospitalized and a second aspiration was done with removal of blood. The role of a bleeding tendency in the etiology of this hemarthrosis was suggested when the patient continued to ooze fresh blood into his dressing for at least 12 hours following this second aspiration.

Physical examination revealed a well developed and nourished young man in considerable discomfort. The temperature was 100° F, pulse rate 90 per minute, respirations 24 per minute, blood pressure, 136/90 mm Hg. The skin showed ecchymoses over both deltoid areas where he had received injections of penicillin. There were no petechiae either before or after application of a tourniquet to the arm. Heart, lungs, abdomen were not abnormal. Liver and spleen were not palpable. The left knee was acutely tender and swollen. It was observed that both hands went into spontaneous intermittent carpal spasm and that the Chvostek sign was strongly positive. There was no pedal spasm. Trousseau's sign was negative. Physical examination was otherwise negative.

Initial laboratory studies were as follows: RBC, 5.40 million; Hb, 16 Gm per cent; WBC, 12,700 per cu mm; polymorphs, 82 per cent; lymphocytes, 17 per cent; monocytes, 1 per cent; platelets, 300,000 per cu mm. Bleeding time, 25 minutes plus (not timed after 25 minutes, but still actively bleeding). Clotting time, 7 minutes (capillary tube method). Serum calcium, 7.36 mg per 100 cc. X-ray of the knee showed evidence of increased synovial fluid.

At this time the nature of his illness was not understood. His knee appeared to have stopped bleeding 24 hours after the aspiration. The acute problem was the presence of tetany. Penicillin therapy was discontinued and he was given calcium lactate 1 gram orally daily and vitamin K (Synkayvite) 50 mg intramuscularly daily.

Further blood studies were then made over the period of his 7 weeks hospitalization. The serum calcium became normal. The only persistent abnormality was the prolonged bleeding time. These studies are discussed more fully below.

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PAST HISTORY

Inquiry into past illnesses revealed that the patient had been a bleeder since about 1 year of age. His first episode of uncontrollable bleeding occurred when he was learning to walk and fell, cutting his lip. He bled for 2 to 3 days despite medical aid. He had always bruised easily, had spontaneous epistaxis, and bled profusely when losing his deciduous teeth. Since childhood he had had 8 to 10 hospital admissions for the numerous bleeding episodes. In recent years, however, there had been a definite diminution in the severity of the abnormal bleeding. He had never been operated upon. There was no history of genito-urinary or previous joint hemorrhage. Following several days of vomiting with sea sickness while in the Navy, he noticed red blood in the vomitus on one occasion. Some years ago he

- MALES
 ○ FEMALES
 ■ MALE BLEEDERS
 × DECEASED

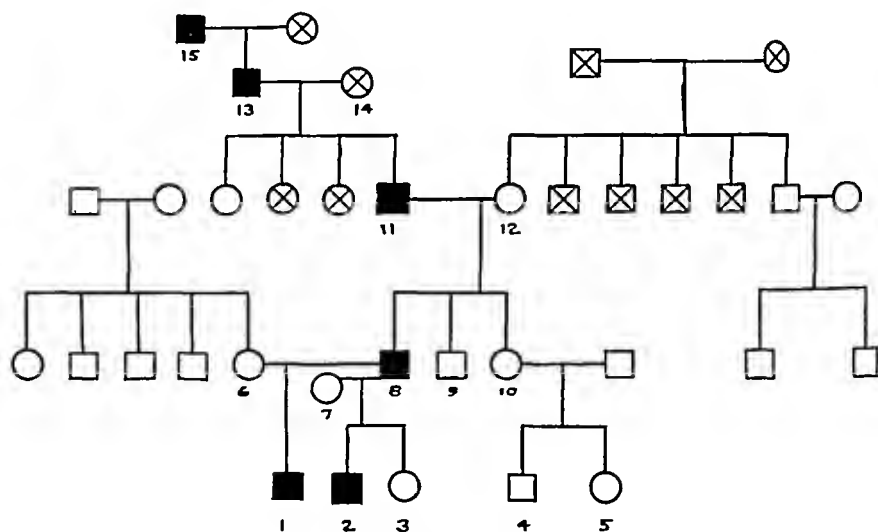


FIG. 1. GENETIC CHART OF PSEUDOHEMOPHILIA

had had intermittent bleeding hemorrhoids, but never noticed more than occasional spotting. Other forms of gastrointestinal bleeding had not occurred. There had been no hemoptysis, even during an attack of mild pneumonia 5 years ago.

The presence of carpal spasm with a low serum calcium brought forth a history of several previous similar episodes when I get excited and during the period of protracted vomiting with sea-sickness.

Dietary history revealed that since childhood he had been a touchy eater but had subsisted mostly on milk, fruits, and meats.

FAMILY HISTORY

One of the most unusual features of this case study has been the family history, for the patient is the fifth of a direct line of males to be a bleeder. This family

history can best be reviewed with reference to the genetic chart. The accessibility and cooperation of the whole family has made this study possible.

No. 1—The patient

No. 2—An 18 month old boy, half brother of the patient, the son of the patient's father and the latter's second wife. His observed bleeding time was 1 hour 10 minutes. His parents reported that he bled for another 2 hours after the test had been performed. On this date his blood count was: RBC, 4.62 million, Hb 15 Gm, WBC, 18,000, P 37, L 57, E 5, M 1. Platelets, 124,000 per cu mm. (Other platelet counts done this same day by the same technician on controls ranged between 120,000 and 180,000.) This child underwent an uneventful circumcision a few days after birth. No history of bleeding episodes prior to this date.

No. 3—An 8 year old girl, true sister of No. 2, half sister of No. 1 (the patient). Bleeding time less than 3 minutes. Blood count and platelets normal. No history of abnormal bruising, epistaxis, bleeding from tooth sockets, nor excessive bleeding following tonsillectomy at age of 6 years.

No. 4—A 3 year, 8 month old boy, first cousin of the patient. Bleeding time 2 minutes 15 seconds. No history of abnormal bleeding. Underwent an uneventful tonsillectomy at 19 months, prior to which operation the bleeding time was tested and reported normal.

No. 5—A 10 year old girl, first cousin of the patient. Bleeding time 2 minutes. No history of pathological bleeding. Uneventful tonsillectomy at age of 4 years, prior to which operation bleeding time was tested and reported normal.

No. 6—The patient's mother, who is living and well. No history of abnormal bleeding at delivery, easy bruising, epistaxis, nor of bleeding after extraction of teeth. Menstrual history normal. She states there was no history of abnormal bleeding in her siblings or her parents. She and the patient's father, No. 8, were divorced several years ago.

No. 7—Stepmother of the patient, the father's second wife, the mother of No. 2 and No. 3. No history of abnormal bleeding.

No. 8—The patient's father, aged 48, also father of No. 2 and No. 3. Definite past history of pathological bleedings, e.g., spontaneous epistaxis lasting 2 to 3 days, easy bruising, bleeding after extraction of teeth lasting 2 to 3 days. His bleeding tendency has diminished in severity in recent years. He has not had a spontaneous nosebleed in about 20 years. Bleeding time 40 minutes. Clotting time 5 minutes (Lee-White method). Clot retraction—complete retraction from edges and bottom of test tube in 1 hour 25 minutes at room temperature. Complete blood count including platelet count, was normal.

No. 9—The patient's uncle, brother of No. 8. No history of pathological bleeding. Had several teeth extracted without undue bleeding. He has no children.

No. 10—The patient's aunt, sister of No. 8 and No. 9, mother of No. 4 and No. 5. No history of pathological bleeding. Deliveries were uncomplicated. Uneventful tonsillectomy 2 years ago. Bleeding time 2 minutes.

No. 11—The patient's paternal grandfather. History of bleeding tendency throughout life with repeated hospitalizations for uncontrollable, spontaneous nosebleeds, bleeding from tooth sockets, and subcutaneous bleeding. Died from a fractured skull with uncontrollable hemorrhage.

No. 12—The patient's paternal grandmother, who has provided the detailed and reliable reports of the lives of those members of the family not now living. She gives no history of abnormal bleeding in herself.

No. 13—The patient's paternal great grandfather, personally known by the grandmother, No. 12. He was a known bleeder who suffered throughout his life with the same easy bruising and spontaneous nosebleeds as did his followers. He died at the age of 56 from a sudden pulmonary hemorrhage following a coughing spell. The existence of pulmonary pathology at the time of death was not determined.

No. 14—The patient's great grandmother, known by the grandmother, No. 12, gave no history of pathological bleeding.

No. 15—The patient's great great paternal grandfather, not known by the patient's grandmother but commonly referred to in the family folktales as having been a bleeder throughout his life. Cause of death unknown.

HOSPITAL COURSE

When first seen, the patient was given calcium lactate orally and vitamin K (Synkayvite) subcutaneously. Penicillin therapy was discontinued. He was given Demerol to control knee pain.

On calcium therapy the tetany and positive Chvostek sign disappeared. Blood calcium level was normal after 3 days of supplemental calcium. This produced no change in the bleeding time.

The blood pressure was measured daily because of an initial diastolic pressure of 90 mm Hg. After about 3 weeks levels of 130/80 mm to 120/70 mm were maintained. Eye grounds were normal. An x-ray of the chest and an electrocardiogram showed no abnormalities. Injections of Synkayvite were discontinued after 16 days as the bleeding time continued to be prolonged despite vitamin K therapy.

The swelling of the knee joint slowly subsided over a period of 3 to 4 weeks. Motion was encouraged. He was allowed up on crutches by the fifth week, and he walked without a cane after the seventh week.

He was discharged from the hospital on the fifty-sixth day. At this time he walked with a slight limp, but had no pain nor tenderness nor swelling in the knee. X-ray of the knee was reported as normal.

THE BLOOD FINDINGS*

1 *The Bleeding Time*—This was determined frequently by one of three selected technicians. With occasional exceptions, the ear lobes were the sites utilized, although finger tips were used occasionally.

It was noted that the bleeding time varied from normal on one occasion only (both ear lobes), to 1 hour and 30 minutes. The majority of bleeding times ranged between 7 and 40 minutes. Variation between 8 minutes and 1½ hours was seen within the same week when done by the same technician. Variation was also seen between the finger and ear lobe bleeding times.

Although varying depths of puncture cannot be avoided in performing bleeding time tests, which may account for variations of a few minutes, the repeatedly prolonged times cannot be attributed to faulty technic when times of over 1 hour were found on more than one occasion. One can safely assume that in this condition the bleeding time was not only prolonged, but that the degree of prolongation varied.

2 *Clotting Time*—On repeated tests the clotting time was between 4 and 7 minutes when done by the capillary glass method, and between 5 and 10 minutes when done by the tube method of Lee and White.

3 *Clot Retraction*—Repeated tests showed good retraction from the walls and bottom of the test tube at 30 to 40 minutes. No liquefaction of the clot was observed after 18 to 24 hours.

4 *Clotting Time of Hemophilic Blood Mixed with Decalcified Plasma of the Patient's Blood* †—The blood of a hemophilic, whose clotting time by the multiple tube

*The author wishes to express to the corpsmen and corpswives of the laboratory of the U. S. Naval Hospital, St. Albans, his appreciation for their interest and help in studying the case presented.

†Done with the aid and cooperation of Dr. A. J. Patch, Columbia University Medical Research Division, Goldwater Memorial Hospital, Welfare Island, New York City.

method of Lee and White at 37° C was 2 hours and 45 minutes, was tested with the plasma of the patient (decalcified by a 5 per cent mixture of potassium and ammonium oxalate) The addition of 0.05 cc of the patient's decalcified plasma to 2 cc of hemophilic whole blood clotted the latter in 19 minutes Normal plasma, similarly treated, clotted the hemophilic blood in 15 minutes

5 *Formed Elements*—Repeated complete blood counts showed the patient to have normal formed elements The red blood count ranged between 4.70 and 5.81 million, hemoglobin between 15 and 16 Gm, white count between 11,000 and 13,000 with normal differential, and platelet counts done on three occasions were 300,000, 236,000, and 156,000 respectively The initial platelet count done when the patient's bleeding time was over 25 minutes was 300,000

6 *Capillary Fragility Test*—Done with a blood pressure cuff midway between systolic and diastolic pressures, this procedure on repeated tests showed rare, scattered, minute petechiae, and was considered normal (negative) The test was done when the patient's blood pressure was elevated as well as when it was normal

7 *Prothrombin Time*—Russell Viper Venom was employed as the source of thromboplastin⁵ The clotting times repeatedly fell within 2 seconds of the control time

8 *Blood Chemistry*—Serum protein determination with albumin-globulin fractionation showed albumin 5.0 grams per cent, globulin 1.8 grams per cent Plasma fibrinogen was 0.32 grams per cent

Bromsulfalein test was normal (less than 5 per cent retention in 30 minutes using 2 mg of the dye per kilo) Serum bilirubin was less than 0.3 mg per cent

Vitamin A absorption curve using Oleum Percomorph as a source of the vitamin was normal

Vitamin C saturation test using 1000 mg of cevalin (Lilly) intravenously gave a normal urinary output in 5 hours (544 mg) The fasting vitamin C plasma level was 0.5 mg per cent

Serum calcium done on admission when there was clinical evidence of tetany was 7.36 mg per cent After 3 days of calcium lactate (15 gr o d) the blood calcium was 10.1 mg per cent, serum phosphorus 4.6 mg per cent, alkaline phosphatase 5.7 Bodansky units, and the clinical signs of tetany were no longer present Oral calcium therapy was discontinued after 10 days Determinations done 1 week after discontinuing calcium therapy were serum calcium 11.2 mg per cent, phosphatase 3.9 Bodansky units Clinical manifestations of hypocalcemia did not recur during the remainder of the patient's hospital course The cause of the initial low serum calcium cannot be readily explained That low blood calcium is seldom if ever a factor in disturbances of the blood clotting mechanism has been indicated by Quick² X-rays of the long bones showed no evidence of demineralization

9 *Capillary Studies*—Following the work of Macfarlane⁶ and his suggestion that the intrinsic physiological disorder in this condition was related to the failure of the capillaries to constrict after trauma, studies on the status of the patient's capillaries were undertaken Under the microscope the capillaries at the nail bed appeared to be normal in distribution and contour After puncturing an isolated loop no constriction of the loop was seen This was done on a finger of each hand

The following photographs of the patient's nail bed capillaries, taken before and after puncture of a capillary loop, demonstrate the failure of constriction of the traumatized, punctured capillary.*

CONCLUSIONS

1. A case of hereditary pseudohemophilia is presented in which the condition was transmitted by and occurred only in the male line. The occurrence of the

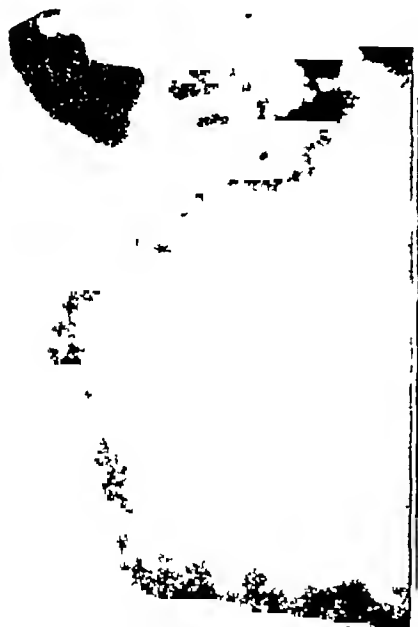


FIG. 2. THE NAIL BED BEFORE RUPTURE



FIG. 3. THE SAME VIEW FOLLOWING RUPTURE OF THE CAPILLARY LOOP IN THE RIGHT HAND FIELD

condition in the male offspring of the patient's father and of the latter's second wife irrefutably proves this point.

2. The degree of prolongation of the bleeding time varied from day to day, and was occasionally normal.

3. Except for transient hypocalcemia with tetany, no defect in the blood elements nor in clot formation was noted.

*Done with the cooperation and technical aid of Dr. A. Wilbur Duryce, Department of Medicine, New York Post Graduate Medical School and Hospital, New York City.

4 There appears to be a failure of normal capillary constriction following trauma This finding confirms the original observations of Macfarlane Whether or not this physiological defect is entirely responsible for the prolonged bleeding time is not proved, but is under further investigation

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PSEUDOHEMOPHILIA

Bj S ESTREN, M D, L SANCHEZ MEDAL, M D, AND
WILLIAM DAMESHER, M D

I INTRODUCTION

THE hemorrhagic disorders can in almost all instances be fairly readily classified as those associated with (1) a deficiency in blood platelets, i e, thrombocytopenic purpura, (2) a disturbance in one of the coagulation factors of the circulating blood, i e, hemophilia, hypoprothrombinemia, fibrinopenia, and (3) a disturbance of the capillary wall, i e, vascular or nonthrombocytopenic purpura. In occasional instances, however, the attempt to classify an example of excessive bleeding becomes difficult. The present report deals with 11 such cases which have been observed during the past five years. These cases have been characterized by an excessive tendency to bleed in the presence of normal platelets, a normal coagulation time, normal serum fibrinogen, and a normal prothrombin time. The only regular abnormality has been a prolonged bleeding time. Similar cases have been previously called pseudohemophilia, and, although this designation leaves much to be desired, we have continued its use chiefly for want of a better title. Recognition of these cases becomes important since excessive bleeding may follow relatively insignificant operative procedures.

Study of the literature of the hemorrhagic disturbances is rendered difficult not only by a lack of uniformity of technic in the performance of certain laboratory tests, but also by a lack of agreement regarding normal values. For these reasons, an analysis of the literature since 1900 and of the routine tests for the study of hemorrhagic disease has been made. The present report deals with the study of 11 cases which we have classified as pseudohemophilia, together with a critical review of the reported cases. A study of the different tests used in establishing the diagnosis of a hemorrhagic disturbance has also been made.

II REPORT OF CASES

Case 1 R. M., an 11 year old white boy, was admitted to the J. H. Pratt Diagnostic Hospital (No 668-) on October 7, 1940, for a study of a tendency to bleed of 6 years' duration.

The patient had had a normal birth and an uneventful ritual circumcision on the eighth day of life without excess bleeding. His parents stated that almost from birth he often developed numerous black and blue spots over the entire body following trauma. Several years before admission a fall on one knee which resulted in an open wound was associated with an undue amount of bleeding. The loss of a deciduous tooth at the age of 10 resulted in oozing from the gums for months. Extraction of a lower tooth 1 week before admission to the hospital was followed by profuse bleeding for 4 hours. Although the clot appeared to be solid, there was continued oozing of blood from its under surface.

There was no family history of bleeding tendency. The past history was irrelevant. Physical examination showed an ecchymosis of the left arm (at the site of an intramuscular injection) and several small ecchymoses near the site of the recent dental extraction, but no frank bleeding. There were no other abnormal findings.

From the Blood Laboratory of the J. H. Pratt Diagnostic Hospital and Boston Dispensary and the Department of Medicine, Tufts College Medical School. Aided by grants from the Upjohn Co., Kalamazoo, Michigan, and the Charlton Fund, Tufts College Medical School.

Laboratory data, which are recorded in table 1, showed a bleeding time of 90 minutes (Duke method). The coagulation time was 14 to 33 minutes at room temperature. Clot retraction was normal. The platelets numbered over 1 million per cubic millimeter. A tourniquet test was strongly positive. Values for prothrombin time and plasma fibrinogen were within normal limits. Further studies have not been made because of the patient's inaccessibility.

Case 2 S. L., a 25 year old white married housewife, was referred for study on May 29, 1941, because of bleeding from the mouth.

The patient had always noted that she bruised easily after trauma, and sometimes spontaneously. Intermenstrual bleeding was frequent. Extractions of teeth at the ages of 5, 18, and 20 were followed by severe bleeding. The gums bled easily and often spontaneously. Two days before referral, she awoke to find blood in her mouth and blood clots about her teeth. She could find no local lesions, but her mouth refilled with clots after the original ones had been removed. One day before she was seen, the same episode recurred, and she continued to bleed from the mouth.

There was no family history of bleeding tendency. The past history, except as noted, was irrelevant. Physical examination revealed small ecchymoses in the popliteal areas but was otherwise unremarkable.

Laboratory data (table 1) showed a bleeding time which was stopped at 4 minutes because of its severity, and which subsequently ranged to 11 minutes (Duke method). The coagulation time was 9 to 15 minutes at room temperature. Clot retraction was normal. Platelets numbered 702,000. A tourniquet test was negative although on a subsequent test it was positive.

Bleeding from the mouth continued intermittently during the next year. Later there seemed to be a regression in bleeding tendency, and the patient was symptom free except for occasional ecchymoses and occasional menorrhagia. At the age of 27 (1943) she contracted rheumatic fever, no bleeding manifestations were present. She has recently developed severe menorrhagia.

Case 3 K. C., a 34 year old white married housewife, was referred for study on May 5, 1943 because of a history of marked bleeding after dental extractions.

The patient had always tended to bleed easily. She had had ecchymoses as a child. At the age of 24, she oozed for 2 weeks after the removal of tonsils and adenoids. She had had difficulty with all dental extractions except one, on each occasion bleeding profusely for 4 or 5 days. Two teeth had been extracted 2 weeks before referral. Bleeding from the sockets was very profuse and prolonged and continued intermittently for about 2 weeks.

The patient's father died at 47 of uncontrollable internal hemorrhage. The father's mother and the father's maternal grandfather bled easily.

Physical examination revealed slightly hyperplastic gums otherwise no abnormalities were noted.

Laboratory findings (table 1) showed a bleeding time of over 21 minutes. The coagulation time was 14 to 22 minutes at room temperature. Clot retraction was slightly delayed. Platelets numbered 1.878×10^6 per cu mm. A tourniquet test was negative. The prothrombin time was normal.

The patient refused further studies and has not been followed.

Case 4 T. C., a 34 year old white Army private, was referred on September 1, 1945, for evaluation of a bleeding tendency.

The patient had been known as a bleeder all his life, having suffered repeated epistaxes and bleeding profusely after slight cuts. There was no family history of bleeding tendency. Physical examination showed no abnormalities.

Laboratory data (table 1) showed a bleeding time of 50 minutes. The coagulation time was 10 to 14 minutes at room temperature. Clot retraction was normal. Platelets numbered 517,000. A tourniquet test was negative. The prothrombin time was normal.

Case 5 R. C., a 27 year old white married woman, was first seen on May 13, 1944 because of menorrhagia over a 3 year period.

Until 3 years before there had been no excess bleeding, easy bruising or menstrual difficulty. Three years before referral, the periods had become unusually profuse and prolonged. In September 1943 an abdominal exploration was performed and an ovarian cyst was removed. Menstrual difficulties, however continued after operation and the patient began to have constant vaginal bleeding. There was also sporadic rectal bleeding. In April 1944 another exploratory laparotomy was attempted but was discontinued because of continuous and excessive oozing from all the cut surfaces. In May 1944 she bled moderately after a dental extraction. In December 1944 because of continued menorrhagia she underwent

TABLE I — Authors Cases of Pseudohemophilia

Case	Sex	Age in years	Family history	Symptoms	Date of observation	Coag. time (mins.)	Bleeding time (mins.)		Clot retraction %	Platelets $\times 1000$	Tourniquet test	Other data
							(Duke method)	(Ivy method)				
R M	M	11	—	Echymoses Bleeding after dental extractions	1940	14-33	90		Normal	1,470	+	Prothrombin normal Fibrinogen 0.62
S L	F	25	—	Echymoses Metrorrhagia Bleeding after dental extractions Gum bleeding	1941 1943	9-15	4 (stopped) 11		Normal	702	once — once	Prothrombin normal
K C	F	34	+	Echymoses Bleeding after dental extractions and tonsillectomy	1943	14-22	over 21		Delayed	1,878	—	Prothrombin normal
T C	M	34	—	Epistaxes Bleeding after cuts Bleeding after operation	8/43 9/43	11 1/2 10-17 24	50 over 31		Normal	517 658	—	Prothrombin normal
R C	F	—7	—	Menometrorrhagia Rectal bleeding Bleeding at operation Bleeding after dental extraction Gum bleeding	5/13/44 5/14/44 6/44	3 8 15 24	5 1/2 2 1/2 and 7 4 1/2 and 6	15 10 1/2	Normal	724	—	
M S	M	—6	+	Bleeding after dental extractions	7/45 8/44 7/45	11 18 23 8 (37°) 9-14 22 19-24 28 10 1/2 (37°)	4 and 4 24 and 9 9 and 11	3 1/2 1 1/2	Normal Normal	765	— +	Asymptomatic
S K	F	34	—	Bleeding after cuts and dental extractions Menorrhagia Peristatic bleeding Ecchymoses	1/45 —/45 6/45 7/45	20-33 16-18 4 6 7 (37°)	10 10 and 9 3 and 3	11	Normal	796	—	Prothrombin normal

TABLE I—Continued

E F	M	14	—	Epistaxes extractions	5/45 7/45	4-9 15-23 29-37 15 (37°)	7 and 15 3 and 6	6 3	Normal	687	—	Prothrombin normal
I W	F	54	+	Bleeding after dental extractions Gum bleeding cuts Menorrhagia	1/46	15-23 14 (37°)	31 and 42	83/1	Normal	450	+	Prothrombin normal Fibrinogen 0.76 Ascorbic acid 0.34 Calcium 10.4
E M	F	23	++	Bleeding after dental extractions Ecchymoses Epistaxes Gum bleeding Bleeding after tonsillectomy	2/46	10-15 1-18 1- (37°)	9 ¹ / ₂ and 18	1 ¹ / ₂	Normal	411	+	Prothrombin normal Fibrinogen 0.48 Ascorbic acid 2.3 Calcium 10.9
N R	F	51	+	Epistaxes Ecchymoses Hematomia Bleeding after dental extractions and operations Bleeding after cuts Postpartum bleeding Postmenopausal vaginal bleeding	5/46	5 ¹ / ₂ 8-9	12 and 12		Normal	644	+	Prothrombin normal Fibrinogen 0.3 Ascorbic acid 0.93 Calcium 10.2

abdominal hysterectomy without excessive bleeding. Thrombin solution and fibrin foam were used at the various suture lines. Pathological examination showed fibromyomata of the uterus with superficial endometriosis of the uterus. In July 1945 vaginal bleeding recurred, but less intensely than previously. In September 1945 slight rectal bleeding recurred. At the same time, there was slight bleeding from the gums.

There was no family history of bleeding tendency. Physical examination was always completely negative.

Laboratory data over a period of years (table 1) showed a bleeding time of 2 to 7 minutes by the Duke method, and 3 to 15 minutes by the Ivy method. The coagulation time varied up to 24 minutes at room temperature. Clot retraction was normal. The platelet count was 724,000 per cu. mm. A tourniquet test was negative.

Case 6 M. S., a 26 year old white man, was referred on August 21, 1944 because of severe bleeding following dental extractions.

He was well until the age of 20, when he bled for 10 days following a dental extraction (starting 3 days after the procedure). At 26 he had a dental extraction without difficulty. A week later, three more teeth were extracted. Four and one half days later, he suddenly began to bleed like a faucet from the site of the extraction and was referred for study.

Five members of the family (grandmother, three uncles, one cousin), all on the paternal side, gave histories of excessive bleeding following operations. Physical examination showed marked caries of the teeth. The findings of early multiple sclerosis involving the extremities were present.

Laboratory data (table 1) showed a bleeding time (Duke) of 24 minutes. The coagulation time was 9 to 22 minutes at room temperature. Clot retraction was normal. Platelets numbered 765,000. A tourniquet test was negative, but was positive on re-examination a year later.

Case 7 S. K., a 34 year old unmarried white woman was first admitted to the J. H. Pratt Diagnostic Hospital (No. 14920) on February 19, 1945, because of right sciatic pain aggravated by motion.

The patient had always had a tendency to bleed. As a child, following a scalp injury which required suturing, she oozed blood from the wound for 2 weeks. Dental extractions at the ages of 12 and 19 years were followed by severe bleeding from the sockets for 3 to 4 weeks. Slight cuts and scratches produced bleeding which lasted for hours. Bouts of ecchymoses were frequent. The menstrual periods were usually profuse. Occasional menorrhagia had been present since the menarche. On October 30, 1944, the patient suddenly developed pain in the right lumbar region and flank radiating into the right groin and accompanied by gross hematuria. The pain disappeared in 6 days, the hematuria in 13 days. On December 15, 1944, she suddenly developed generalized ecchymoses which faded only after several weeks. On January 1, 1945, she experienced severe pain at the right iliac crest, radiating down the posterior thigh and calf to the right ankle, aggravated by motion and relieved by flexing the knee and thigh on that side.

There was no family history of bleeding tendency. Physical examination showed a few small telangiectases of the face and upper lip but none elsewhere. There was limited straight leg raising on the right with tenderness along the sciatic nerve. The rest of the examination was negative.

There was slow recovery from what appeared to be a hemorrhage in the right psoas muscle. The patient was discharged for convalescence on February 24, 1945. In April 1945 she returned for dental extraction. No signs of the previous neurologic disturbance were present. Two teeth were removed. No immediate bleeding followed, but, a few hours later, profuse bleeding began from the tooth socket and continued for 6 days despite the use of gauze packing, epinephrine, thrombin and fibrin foam, and the administration of two transfusions of whole blood. At this time the gum was sutured, oozing continued but no further frank bleeding occurred and the patient was discharged. On June 18, 1945 the patient was readmitted for profuse vaginal bleeding, which began a few days earlier than the expected menstrual flow. There was no associated pain. The hemorrhage continued for 7 days despite a transfusion of blood, the administration of calcium, and local packing, it then stopped spontaneously. Since then the patient has been free of hemorrhagic disturbance, but has shown a moderate hypochromic anemia. It is of interest that she believes that the hemorrhagic episodes of October 1944 and June 1945 were precipitated by taking iron for anemia. She therefore refuses to take iron for the present anemic state.

The laboratory findings at various times are recorded in table 1. The bleeding time varied from 3 to 13 minutes by the Duke technique and was 11 minutes by the Ivy method. Coagulation time varied from 6 to

33 minutes at room temperature, and was 7 minutes at 37° C. Clot retraction was normal. The platelets numbered 796,000 per cu. mm. A tourniquet test was negative. Prothrombin time was normal.

Case 8 E F, a 14 year old white boy, was referred on May 23, 1945, because of bleeding after dental extraction. The patient had always had frequent nosebleeds. At the age of 9 years, he bled severely and excessively following dental extraction. At 14 years he bled continually for 3 days following dental extraction and was therefore referred for study.

There was no family history of bleeding tendency. Physical examination showed no abnormalities.

Laboratory data (table 1) revealed a bleeding time up to 15 minutes (Duke). Coagulation time was up to 29 minutes at room temperature, and 15 minutes at 37° C. Clot retraction was normal. The platelets numbered 687,000 per cu. mm. A tourniquet test was negative. The prothrombin time was normal.

Case 9 F W, a 54 year old white Finnish born housewife, was admitted to the J. H. Pratt Diagnostic Hospital (No. 17679) on January 19, 1946, because of anemia and a long history of a tendency to excessive bleeding. At the age of 6 years the patient bled severely following the removal of several deciduous teeth. In the following years a number of dental extractions were followed by severe hemorrhage lasting from several hours to as long as 3 weeks. Spontaneous bleeding from the gums was occasionally present and bleeding from minor cuts was always marked and prolonged. Menarche occurred at 16 and menstruation occurred regularly every 26 days, the duration of flow was 5 to 14 days, occasionally as long as 28 days. On three occasions hospitalization was required for excessive menstrual bleeding. Menopause occurred at 47, and she had had no vaginal bleeding since. In May 1945 at the age of 53, the patient vomited brownish material and fainted. X-rays of the stomach were negative. No further such episodes occurred. Epistaxes, ecchymoses, and hematuria had never been present. The patient had never become pregnant. A fracture of the humerus at the age of 49 was not followed by excessive hemorrhage. Iron therapy for anemia had been given for several years.

Two brothers and three sisters were severe bleeders. One of these brothers has a son aged 10 who has bled severely following dental extractions. The patient's father was a bleeder, as were his sister and possibly (to a lesser degree) his three brothers. The patient's paternal grandmother was also a bleeder. The patient's mother and mother's family showed no bleeding tendency. It is of interest that the patient's family come from an island off Finland, where von Willebrand's original cases of pseudohemophilia were found.

Physical examination was essentially negative with the exception of a moderately loud systolic murmur at the apex transmitted toward the base. No telangiectases, petechiae, or ecchymoses were present. The liver, spleen, and lymph nodes were not palpable.

Laboratory data (table 1) showed a bleeding time of 31 minutes in one ear, 42 minutes in the other ear, and 8¾ minutes by the Ivy method. The coagulation time was 23 minutes at room temperature and 14 minutes at 37° C. Clot retraction was normal. The platelets numbered 450,000 per cu. mm. A tourniquet test was markedly positive. Values for prothrombin time, plasma fibrinogen, calcium, and ascorbic acid were within normal limits. Examination of the capillaries of the finger nail bed showed essentially normal capillaries with some tortuosity of some of the proximal loops.

Case 10 E M, a 23 year old white female bookkeeper, was referred on January 30, 1946, because of continued bleeding following dental extractions.

Each dental extraction from the age of 6 years had been followed by an excessive amount of bleeding which started immediately after the operation and lasted for several days to several weeks. Such episodes occurred at the ages of 6, 16, 21, 22, and 23. The patient had always bruised easily, but never spontaneously. As a child she had daily spontaneous epistaxes lasting up to one half hour, more recently epistaxes had been infrequent. The gums bled very easily but only with brushing of the teeth. There had never been excessive bleeding after cuts. Menarche occurred at 12 and periods occurred irregularly (every 4 to 8 weeks), and lasted only 4 days, with severe flow only on the second day. Removal of the tonsils and adenoids at the age of 21 was followed by recurrent bleeding for about a month. A week before consultation, she had had six teeth removed by an oral surgeon. Bleeding was marked during and after the procedure, and continued to the time of examination. Blood came like a faucet and continued to well up despite packing of the sockets.

The patient's father had had severe epistaxes as a child, but there was no other history of bleeding tendency in the parents' grandparents, or siblings of the patient. Physical examination revealed a healthy-

appearing young woman with positive findings restricted to the mouth. Here at the site of recent extractions, oozing of blood was noticeable about the gauze packing. Most of the teeth were carious. Liver, spleen, and lymph nodes were not palpable.

Laboratory data (table 1) revealed a bleeding time (Duke) of 9½ minutes in one ear and 18 minutes in the other ear. The Ivy bleeding time was 1½ minutes. The coagulation time was 10 to 18 minutes at room temperature, and 12 minutes at 37° C. Clot retraction was normal. The platelets numbered 411,000 per cu mm. A tourniquet test showed 23 petechiae (positive). Values for prothrombin time, plasma fibrinogen, calcium, and ascorbic acid were within normal limits.

Case 11. N. R., a 51 year old white housewife, was admitted to the J. H. Pratt Diagnostic Hospital (No. 18633) on May 7, 1946, with the chief complaints of fatigue and exhaustion.

During the course of the investigation, it was found that the patient had always had a tendency to bleed. From childhood on, epistaxes, spontaneous ecchymoses, and spontaneous subcutaneous hematomata were frequent. Slight cuts and traumata were always followed by severe hemorrhage. At the age of 7 years, removal of tonsils and adenoids was followed by unusually severe bleeding. Loss of teeth during childhood was accompanied by prolonged hemorrhage from the tooth sockets. Extraction of teeth in childhood and during adult life was invariably followed by profuse hemorrhage, the most recent occurring at the age of 31 years and lasting for 2 weeks. The menstrual periods were regular but very profuse. At the age of 33 curettage was undertaken because of menorrhagia for 3 months and revealed no local lesion. Menopause occurred at the age of 48 but two spontaneous vaginal hemorrhages similar in all respects to normal menstrual periods except for their shorter duration (3 days instead of her normal 6), had occurred twice since menopause and were not attributable to any local lesion. At the ages of 33 and 34, the patient was delivered of normal children with marked post partum hemorrhage which was so intense on the first occasion that she nearly bled to death.

The patient's mother had had spontaneous epistaxes and ecchymoses from childhood until death at the age of 73 of myocardial infarction. In addition, menorrhagia was frequent and post partum hemorrhage always marked. The mother had four sisters and two brothers, none of whom had excessive bleeding. The mother's parents also did not have excessive bleeding. The patient had one sibling, a brother aged 48, who also had frequent epistaxes, ecchymoses, and hematomata. At the age of 4 years, this brother underwent removal of tonsils and adenoids with profuse postoperative hemorrhage. This brother has one daughter who shows no bleeding tendency. The patient's two children, a son aged 15 and a daughter aged 17, both show a tendency to hemorrhage. The son bled profusely after cuts as a child, had frequent epistaxes, bled after removal of tonsils and adenoids at the age of 5 years, and has bled after extraction of teeth since the age of 17. A daughter of this son shows no bleeding tendency. The patient's daughter bled profusely after removal of tonsils and adenoids at the age of 5 years and has profuse menstrual bleeding but shows no other tendency to hemorrhage.

Physical examination showed slight enlargement of the heart. The blood pressure was 110/120. Psychiatric examination resulted in a diagnosis of manic-depressive psychosis. Laboratory data (table 1) showed a bleeding time of 12 minutes in each ear (Duke). The coagulation time was 5 to 8 minutes at room temperature. Clot retraction was normal. The platelets numbered 644,000 per cu mm. A tourniquet test was markedly positive. Values for prothrombin time, plasma fibrinogen, calcium, and ascorbic acid were normal.

III. HISTORICAL ASPECTS

Before the second decade of this century, there was little attempt in the voluminous literature on hemorrhagic diseases to study cases in detail in an effort to segregate various entities. The early reports of Austin and Pepper¹ and of Hess, although complete in some respects, are unfortunately lacking in some of the tests which are today considered essential in evaluating a tendency to bleed. Glanzmann in 1918² was the first to report in detail a series of patients with what appeared to be an atypical hemorrhagic diathesis.

Glanzmann studied three families with a total of 49 members and found that 12 of 23 males and 21 of 26 females were bleeders. Some of his cases were typical

examples of idiopathic thrombocytopenic purpura. The other cases were characterized by several common factors

- 1 They all bled excessively
- 2 The bleeding time was normal
- 3 The coagulation time was normal
- 4 The platelet count was usually normal
- 5 The clot retraction was abnormal (poor, delayed, incomplete)
- 6 The platelets were qualitatively abnormal (no granules, pyknotic granules, peculiar shapes and sizes)

Because he believed that the excessive bleeding was due to a platelet defect, Glanzmann named this disorder hereditary hemorrhagic thrombasthenia. His cases are commonly referred to as typical examples of pseudohemophilia and are often considered as identical with those of von Willebrand.²³ The existence of such cases has, however, never been confirmed and no similar cases have been reported.

Further sporadic cases of peculiar forms of excessive bleeding continued to be reported in the literature under various designations. Rosenfeld⁴ described two siblings with hemarthrosis, normal clotting time, and excessive bleeding time. In one, a reduction in platelets may have been of significance, but there was no such abnormality in the other, and the clinical pictures were identical. Roskam,⁵ Isch-Wall,⁶ Rosenthal,⁷ and others reported atypical hemorrhagic syndromes during the next few years. In 1928, articles on atypical bleeding appeared simultaneously from several parts of this country.⁸⁻¹² In one of these reports, Minor⁹ pointed out that his own cases were neither idiopathic thrombocytopenic purpura nor hemophilia, yet were quite different from the syndrome described by Glanzmann in 1918, thus intimating the existence of yet a different bleeding disorder.

Further reports, some complete, many grossly inadequate for critical evaluation,¹³⁻²² continued to appear at frequent intervals in the literature. Under the designations of hereditary hemorrhagic diathesis, hereditary hemorrhagic thrombasthenia, pseudohemophilia, and thrombasthenic purpura, various investigators reported cases of unexplained excessive bleeding with no constant abnormality except for an increase in the bleeding time. Finally, in 1933, von Willebrand²³ published a comprehensive review including details of 23 cases of his own.

Von Willebrand's contribution was great. He had previously²⁴⁻²⁵ published fragments of data on several of his cases, but had never compiled complete data until 1933. His original patients derived from a single family living on the Åland Islands off Finland, in which 23 of 66 individuals showed a bleeding tendency. Many of his cases lacked adequate laboratory studies, and only 7 of them included sufficient data to warrant their designation as definite cases of pseudohemophilia. Von Willebrand pointed out that his cases, as well as those of most other German authors and of virtually all American authors, belonged to a single group which was distinct and separate from the cases reported by Glanzmann.

Since 1933 further reports of a similar bleeding tendency have continued to appear.²⁶⁻³⁶ In recent years there has been a tendency to include data on all known hemorrhagic tests, so that few cases must be discarded for want of adequate investigation.

TABLE 2—Summary of Cases of Pseudohemophilia in the Literature since 1900

Author and year	Name given to disease	Case No	Sex	Age (Yrs)	Family history	Symptomatology	Coagulation time (mins)	Bleeding Time (mins)	Clot Retraction	Platelets $\times 1000$ per cu mm	Tourniquet test	Remarks
Rosenfeld 1921 (4)	Idiopathic purpura with unusual features	1	M	11	+	Polyarthritides Easy bruising Purpura Probable hemorrhage into joints Hematemesis	Normal	10	?	98	?	This case is identical with case 2 his sibling and there is no evidence that the apparent reduction in platelets was significant
Roskam 1923 (5)	Purpura	2	M	15	+	Jaundice at age 5 Polyarthritides since age 5 Hematuria Purpura Hemarthrosis	Normal	10	?	204	?	
Isch Wall 1926 (6)	Purpura	3	F	16	?	Epistaxis Menorrhagia	7½	12	?	241	?	
	Hemogenic (Werlhol's disease)	4	F	21	?	Epistaxis and gum bleeding since 3½ years Ecchymoses Menorrhagia Hemoptysis	4	16 to over 60	Normal	Over 200	?	Platelets once 130 000
Rosenthal 1927 (7)	Chronic thrombasthenic purpura	5	F	?	+	Epistaxis Ecchymosis Purpura	26	6	Normal	180	—	Mother of case 6
		6	M	?	+	Epistaxis Ecchymosis Purpura	16	10	Normal	240	—	
Giffin 1928 (8)	Unusual hemorrhagic disease	7	F	9	—	Epistaxis since 14 mos Purpura Ecchymosis Bleeding from gums No excess bleeding from cuts Later menorrhagia Hemorrhage after tooth extraction Excess bleeding from splenectomy wound	Preop 5 Postop 7	Pre 60 Post 120	Absent	Pre 300 Post 272-352	?	Data in Kennedy (10) Splenectomy done with no improvement
Minot 1928 (9)	Familial hemorrhagic condition with prolonged bleeding time	8	M	33	+	Severe bleeder since childhood	Normal	NL-60	?	Increased	—	
		9	M	?	+	Severe bleeder since childhood	Normal	NL-120	Normal	Increased	—	
		10	M	?	+	Bleeder all his life	Normal	NL-30	?	Normal	—	
		11	M	?	+	Ecchymoses all his life	Normal	NL-60	?	Normal	—	
		12	F	2	+	Epistaxis Purpura	Normal	NL-45	?	Normal	—	
Kennedy 1928 (10)	Nonthrombopenic purpura	13	F	7	?	Ecchymoses Bleeding from gums	Preop 12 Postop 7	Pre 28 Post 28	None	Preop 1447 Post 242	?	Splenectomy with good clinical results but bleeding time unchanged

Little & Lyre 1928 (11)	Infamilial bleeding tendency of an unusual type	14 F	13	+	Bleeder all her life via nose uterus joints retron	4-6	30-170	?	280-100	?	Death 9 hrs after splenec- tomy Autopsy—abdomen contained free blood although pedicle well ligated No bleed- ing points found His sister of same 11 Spleen palpable
Buckman 1928 (12)	Atypical patho- logical hemor- rhage in early life	15 F	11	+	Bleeding via nose gums joints	1	Greatly prolonged	?	350-370	?	
		16 F	3	+	Epistaxis Ecchymosis Excess bleed- ing after cuts	10-15	15-30	Normal	500 680	?	
		17 M	9 mos	-	Epistaxis Petechiae	Normal	18-25	?	Normal	?	
		18 M	3	-	Epistaxis Ecchymosis	6	20-60	?	Increased	?	
		19 M	1	+	Epistaxis Ecchymosis	Normal or slight pro- longed	Prolonged	?	300 600	?	
Roskam 1929 (13)	Werthof's disease	20 F	6 mos	-	Ecchymosis Intracranial hemorrhage	10	Over 30	?	500	?	Clotting time—10 mins by capillary tube method Died of cerebral hemor- rhage
		21 F	12½	++	Tendency to severe hemorrhage since infancy ecchymoses epistaxis Bleeding from mouth and ear Vagi- nal bleeding at 8 yrs Bleeding after dental extraction Bleeding from nose and gums Profuse menstrua- tion	8	8 105	Normal	Over 350	+	
Rosling 1929 (14)	Hereditary hemor- rhagic diathesis	22 F	20	+	Bleeding since childhood via nose and gums Easy bruising Menorrhagia Excess bleeding from cuts	1½	Over 30	Normal	210-590	-	
Rothman and Nixon 1929 (15)	Hereditary hemor- rhagic thrombo- asthenia	23 M	13	+	Epistaxis Ecchymosis Easy bruising Bleeding from gums Bleeding after tooth extraction	3-8	6½-35	Ab-ent and normal	700-413	-	
		24 M	5½	+	Epistaxis Petechiae nt lips Telangi- ectatic cheeks	6	7½	?	127	-	Spleen palpable This pt and No 25 live platelet counts by a dry smear technic considered nor- mal by the author
Curschmann 1930 (16)	Pseudothrombophilia	25 M	54	++	Epistaxis	4	12	?	126	-	

TABLE 2 (Continued)

Author and year	Name given to disease	Case No	Sex	Age (Yrs)	Family history	Symptomatology	Coagulation time	Bleeding Time (Vlms)	Clot Retraction	Platelets $\times 1000$	Tourniquet test	Remarks
Morawitz and Jürgens 1930 (17)	? Thrombasthenia	26 M	M	16	-	Bleeding since age 4 via nose, gums joints skin kidneys Petechiae	8-18	35 to over 45	Normal	480-560	+	
Jürgens and Nau mann 1931 (19)	? Thrombasthenia	27 M	M	21	?	Bleeding via skin mucosae lungs	9-12	Over 50	?	200	+	
Stuber and Lang 1931 (20)	Thrombasthenia	28 F	F	31	+	Gastrointestinal bleeding since age 5 Menorrhagia Epistaxis Easy bruising Hematemesis	9	8½	?	400	?	
Kugelmass 1932 (21)	Hereditary thrombasthenia purpura	29 M	M	6	?	Hemorrhages into joints	11	18	Normal	Normal	+	Diagnosis was hemophilia with athrombogenic purpura with hemophilic joints Splenectomy with continued excess bleeding Blood transfusions helped
von Willebrand and Jürgens 1933 (23)	Constitutional thrombopathy (pseudohemophilia)	30 T	T	10	+	Epistaxis and purpura since 1 year	10	15	Absent	350	-	
		31 M	M	19	+	Epistaxis	10-14	12	Normal	141-143	-	
		32 F	F	13	+	Bleeding via nose and gums Petechiae Easy bruising	8-11 and 13-25	Over 60	Normal	110-540	+	No correlation of platelet level with bleeding time
		33 F	F	33	+	Past tendency to bleed	14-16	28	Normal	440	+	
		34 F	F	77	+	Epistaxis Hematemesis Easy bruising	8-12	18	Normal	254	+	
		35 F	F	17	+	Epistaxis Easy bruising	14-21	64	Normal	342	+	
		36 F	F	52	+	Epistaxis Metrorrhagia Ecchymosis	8-25	Greatly increased	Normal	112-180	+	No correlation of T T with level of platelets
Weeks 1934 (26)	Familial bleeding	37 T	T	10	?	Bleeding via nose and gums	9-15	9	Normal	192	+	
		38 M	M	13	+	Epistaxis since 18 months Ecchymosis Bleeding from gums Probable hemarthrosis	6	16-180	Normal	Normal	?	Spleen just palpable Right ankle 'similar to that seen in hemophilia'
		39 M	M	10	+	Epistaxis Easy bruising	13	Over 60	Normal	Increased	-	

Author and Year	Disease	Age	Sex	Onset	Course	Treatment	Prognosis	Platelet Count	Spleen	Other
Bailey and McMillan 1935 (27)	Familial purpura	40	M	+	Bleeding since 18 mos. via nose and gums. Tarry stools. Ecchymosis 2 petechiae in left conjunctiva once.		?	190-380	?	One platelet count 40,000 not correlated with bleeding time. Negro.
		41	M	+	Bleeding via nose and gums. Tarry stools. Hematuria.		?	169-260	?	
		42	M	+	Bleeding via nose and gums. Rectal bleeding. Melena. Hematemesis.		+	290-960	+	Spleen palpable.
		43	T	+	Easy bruising since infancy. Epistaxis. Ecchymosis. Menorrhagia.		+	Normal	+	
Lowler 1937 (28)	Hereditary pseudo hemophilia	44	M	+	Epistaxis since age 10. Bleeding from gums. Melena.		+	410	+	
		45	M	+	Ecchymosis. Epistaxis since age 13. Excess hemorrhage after cuts and dental extraction.		+	120	+	
		46	F	+	Epistaxis. Easy bruising. Excess bleeding from cuts.		+	450	+	
		47	T	+	Ecchymosis. Epistaxis. Excess bleeding from cuts since childhood. Menorrhagia. Severe postpartum bleeding.		?	312	?	
Schlicke and Hall 1938 (30)	Hereditary pseudo hemophilia	48	T	-	Excess bleeding, after tonsillectomy and dental extraction. Menorrhagia (? local origin).		?	315	?	
		49	F	+	Excess bleeding after injury as child. Menorrhagia. Epistaxis during pregnancy. Postpartum bleeding.		+	10-707	-	Spleen enlarged 21 on one occasion.
		50	M	+	Easy bruising. Bleeding from gums. Bleeding via nose and gums since childhood. Rare ecchymosis. Excess bleeding after cuts.		+	188	-	X-ray to spleen improved.
		51	M	+	Bleeding via nose and gums since childhood. Rare ecchymoses. Excess bleeding after dental extraction at 16.		+	187	-	
Ceiger and Evans 1938 (29)	Hereditary hemophiloid purpura with prolonged bleeding time	40	M	9						
		41	M	15						
		42	M	33						
		43	T	22						
Hain 1939 (41)	Hereditary pseudo hemophilia	44	M	51						
		45	M	27						
		46	F	22						
		47	T	33						
Braun 1939 (42)	Hereditary pseudo hemophilia or hereditary hemorrhagic diathesis	48	T	33						
		49	F	35						
		50	M	32						
		51	M	40						

TABLE 2 (Continued)

Author and year	Name given to disease	Case No.	Sex	Age (yrs.)	Family history	Symptomatology	Coagulation time	Bleeding time (mins.)	Clot Retraction	Platelets $\times 1000$	Tourniquet test	Remarks
Carpenter and Allen 1940 (33)	Chronic agnogenic hemorrhagic disease	52 F	F	4	+	Epistaxis Easy bruising	3½	20	?	204	+	No improvement with vitamins K C Pp citrin Prothrombin 37% increased to 100% with vitamin K but with out change in complaints or findings No improvement with vitamin C
		53 M	M	27	-	Normal until 22 yrs since then bleeding via nose gums and into skin	Normal	Over 30	Normal	150-233	+	
		54 F	F	30	-	Echymoses and excess bleeding after cuts since childhood Epistaxis Menorrhagia Bleeding after dental extraction	Normal	Over 30	Normal	120-250	+	
Smith and Watkins 1941 (34)	Hereditary pseudohemophilia	55 F	F	28	-	Echymosis Profuse bleeding after operations and dental extractions Menorrhagia	Normal	Over 30	Normal	210-235	+	Blood ascorbic acid 0.6 Gm increased to 2.1 Gm by vitamin C therapy with concomitant reduction in bleeding time from 30 to 6½ but without change in symptomatology
		56 M	M	30	+	Bleeding as child via nose and gums Melena Excess hemorrhage after removal of impacted tooth Cus tritis	8	6½ to over 30	Normal	260	+	
		57 F	F	?	-	Tendency to bleed	9	Over 60	Normal	260	-	
MacFarlane (35)	Pseudohemophilia	58 F	F	?	+	Tendency to bleed	2½	24	Below NI	395	+	
		59 F	F	?	+	Tendency to bleed	9	Over 120	Normal	320	+	
		60 F	F	?	+	Tendency to bleed	14	7½	Normal	352	+	
		61 F	F	?	+	Tendency to bleed	12	Over 20	Normal	385	+	
Cronkite and Lerner 1944 (36)	Hereditary hemorrhagic thrombasthenia	62 M	M	?	+	Excess bleeding after tonsillectomy dental extractions and small cuts Severe bleeding following a hemorrhoidectomy	2-4	4½ 7½	Normal	178-356	-	

Figures in parentheses refer to bibliography listing

? = no statement made

Table 2 records 62 cases which we regard as definite examples of pseudohemophilia and which have appeared in the literature since 1900. The criteria for admission of cases into this category are stated below.

IV ANALYSIS OF CASES INCLUDING THOSE IN THE LITERATURE

An analysis of the clinical and hematologic data in our series of 11 cases has been combined with a review of those cases in the literature which fit the criteria we have established for the diagnosis of pseudohemophilia. The literature on hemorrhagic disease is often quite sketchy, with many reports dealing with excess bleeding after cuts, hereditary epistaxis, etc., without adequate laboratory studies. It has, therefore, been necessary to limit detailed consideration to those cases with relatively complete studies. Cases of hemorrhagic disease were arbitrarily classified into the following groups:

- Group O Cases of definite hemophilia or thrombocytopenic purpura
- Group I Cases with insufficient data to allow a definite diagnosis
- Group II Cases with normal laboratory data, and testing done only once
Cases in Groups O, I, II were discarded from further consideration
- Group III Cases of pseudohemophilia. Sixty-two cases which we considered as adequately studied were selected for inclusion. They were selected as follows:
 - (a) Tendency to excessive bleeding
 - (b) Adequate study (at least one determination each of bleeding time, coagulation time, number of platelets, plus an adequate clinical history)
 - (c) A definitely increased bleeding time in the presence of usually normal blood platelets and a usually normal coagulation time

Family History

The cases in the literature show a predominantly positive family history of a tendency to bleed. A statement in this regard was recorded in 57 cases, of these, 44 (77.2 per cent) had some familial history of bleeding, and 3 more (5.3 per cent) had a probable familial history. In our own 11 cases, 4 patients had a definite history of familial bleeding, and in a fifth a questionable family history was elicited.

This marked familial incidence has resulted in the usual title of hereditary pseudohemophilia or hereditary hemorrhagic tendency. Several investigators, notably von Willebrand,²³ consider that the disease is due to the transmission of a sex-linked dominant hereditary gene. However, sporadic cases are fairly common, and the diagnosis of pseudohemophilia must be made in many cases with a completely negative family history of bleeding.

Sex and Race

Of the 62 cases in the literature, 33 were females, and 29 were males. Of our 11 cases, 7 were females and 4 males. There is thus a slight predominance of women (54.8 per cent of the total series), but this is not statistically significant. The

important fact is that pseudohemophilia occurs in both males and females and is transmitted through either sex.

Two cases of pseudohemophilia are reported in Negro brothers.²⁷ All other cases occurred in white individuals.

Symptomatology

The cardinal symptom of pseudohemophilia is excessive bleeding from the nose, the gums, the gastrointestinal tract, the uterus, into the skin, the urine, and the joints. Although bleeding is at times completely spontaneous it usually occurs after slight cuts or trauma. Postoperative bleeding is common and marked. Hemorrhage is often inconstant, at times occurring with one operation but not with another. Cycles of increased bleeding may occur, with marked hemorrhage at some times and complete normality at others.

TABLE 3 — Sites of Bleeding in 64 Cases of Pseudohemophilia

Nose (epistaxis)	48 (75.0%)
Skin (ecchymosis, petechiae)	45 (70.3%)
Gums	25 (39.1%)
Postoperative, including after dental extraction	20 (31.2%)
Gastrointestinal tract	8 (12.5%)
Joints	8 (12.5%)
Kidneys (hematuria)	4 (6.3%)
Lungs (hemoptysis)	2
Retina	1
Perineural	1
Intracranial	1
Uterus	20 (76.9% of females aged 12 or more)

No sites of bleeding were recorded in cases 8, 9, 10, 33, 57, 58, 59, 60, 61 (table 2).

Of the 73 cases, sites of bleeding were recorded in 64. No sites were listed for cases 8, 9, 10, 33, 57, 58, 59, 60, 61 (table 2). These sites are listed in table 3. Several points are of interest.

1. In the entire group, *epistaxis* was the most prominent symptom, occurring in 75 per cent of the cases. The bleeding was usually spontaneous, although perhaps usually more marked when precipitated by trauma. It was most intense during childhood, became less frequent and less severe in adult life, and in some cases completely disappeared. Four of our own 11 cases (T. C., E. F., E. M., N. R.) had epistaxes. In one of these cases bleeding had occurred almost daily at certain times and had lasted as long as 30 minutes. By the age of 23, however, epistaxis was infrequent and of short duration.

Some cases of familial epistaxis¹⁶ and certain hitherto unexplained cases of epistaxis not associated with local lesions, arteriosclerosis, or hypertension may belong in this category.

2. *Bleeding into the skin* occurred in 70.3 per cent of the total number of cases, and in over half of our own group. Usually, this symptom was manifested as easy bruising, but spontaneous ecchymoses were also common. Petechiae were rare.

Curschmann noted dilated capillaries on the face of his first patient,¹⁶ and one of our patients (S. K.) presented the same findings. These vessels did not bleed, and no other telangiectases were noted in either instance in patient or family; it is therefore improbable that the dilatation was related to the syndrome of bleeding.

3 *Bleeding from the gums* was frequent, occurring in 25 of the total number of cases (39.1 per cent) and in 4 of our own group (36.4 per cent). In some cases this was spontaneous, in others, it occurred only on brushing the teeth. No particular local pathology was present to account for the bleeding.

4 *Dental extraction* often gave the first indication of pseudohemophilia. Excessive bleeding occurred with dental extraction in each of our 11 cases, and in 5 it was the immediate cause for hematologic investigation. Hemorrhage under such circumstances occurred in two general forms: (a) during the extraction, and continuing indefinitely despite treatment, and (b) several hours after the extraction, starting as profuse oozing from the socket, then becoming uncontrollable despite pressure and various types of local therapy. Ultimately the bleeding stopped, although transfusions were occasionally required to combat the often severe post-hemorrhagic anemia.

5 Severe *postoperative bleeding* of other types was also found. In Little and Ayres' first case,¹¹ death occurred 9 hours after splenectomy. Autopsy disclosed an abdomen full of blood which apparently had oozed from the blood vessels, since the ligatures had been well placed and effective. In Cronkite and Lozner's report²⁷ it was not until the patient continued to bleed after hemorrhoidectomy that he was discovered to have bled similarly after tonsillectomy, dental extraction, and small cuts. In one of our cases (R. C.) it was necessary to discontinue an abdominal operation because of excessive hemorrhage, but later the patient underwent a similar procedure uneventfully.

Not all patients with pseudohemophilia bled after cuts or operations, and the same patient might bleed at one operation and not at another. Evidently there was no correlation between the hemorrhagic tests and the occurrence of postoperative hemorrhage.

6 *Bleeding into the joints* was recorded in 8 cases in the literature, 1 c, in 12.5 per cent of the total cases. Details of the pathologic changes in the joints were usually not recorded. In Kugelmass's first case²¹ the joints were reported as typical of those found in hemophilia. In Weeks's first case²⁶ an ankle joint was similar to that seen in hemophilia.

7 *Uterine hemorrhage*, in the form of menorrhagia or metrorrhagia, occurred frequently and was found in 20 (76.9 per cent) of the 26 women above the age of 12 years. Uterine or vaginal hemorrhages did not occur at a younger age except in Roskam's patient,¹³ in whom vaginal bleeding occurred at 8 years and menorrhagia after menarche. The symptom took no special form, but presented itself as unduly profuse, prolonged, or repeated hemorrhage. In some patients (cf. F. W.) menstrual bleeding was of such severity as to necessitate hospitalization and transfusion.

8 Less common manifestations of hemorrhagic tendency in pseudohemophilia were gastrointestinal bleeding, hemoptysis, hematuria, retinal bleeding, perineural bleeding, and intracranial hemorrhage. S. K. in our group of cases developed gross hematuria and signs of sciatic nerve irritation as a result of bleeding.

Laboratory Data

1 *Bleeding Time*—By definition, this was always increased. It varied from normal to over 300 minutes in the recorded cases, and from $2\frac{1}{2}$ to 90 minutes in our own cases. In many instances, bleeding continued unabated from the site of puncture for hours and prolonged pressure was necessary to stop the bleeding.

The bleeding time was often extremely variable, being greatly prolonged at one time, and only slightly increased or even normal at other tests. Minot described a variation from normal to 2 hours (case no. 9*), Rosham (no. 21) from 8 minutes to 105 minutes, Bailey and McAlpin (no. 40) from $1\frac{1}{2}$ minutes to over 35 minutes, Fowler (no. 42) from $3\frac{1}{2}$ minutes to over 5 hours, Smith and Watkins (no. 56) from $6\frac{1}{2}$ minutes to over 30 minutes. In our own group, in S. K., the Duke bleeding time was 10 minutes on some occasions, and only 3 minutes at another time, although on the latter occasion uterine bleeding was uncontrollable. This marked variation makes it impossible to evaluate the many reports in which the bleeding time was normal on the single occasion on which it was measured.

There is some indication that the Ivy bleeding time determination may show abnormalities when the Duke bleeding time is normal (case R. C.) and vice versa (case M. S., table 1). Because of these variations, both procedures have been carried out, prolongation of either or both being taken to be abnormal.

2 *Coagulation Time*—It is difficult to evaluate the normal for coagulation time determinations in the literature. We have, therefore, separated four categories:

- (1) Normal Patients reported as normal, and those in whom the Lee-White coagulation time did not exceed 16 minutes. This figure was chosen arbitrarily from our controls.
- (2) Borderline Patients with Lee-White coagulation times over 16 but not over 20 minutes.
- (3) Abnormal Patients with Lee-White coagulation times over 20 minutes. Included also is Buckman's last patient (no. 20, table 2) in whom the coagulation time was measured by the capillary tube method and was definitely prolonged.
- (4) Variable Cases with a normal coagulation time on one occasion, and borderline or abnormal on others.

Fifty-three of the 62 cases in the literature (85.5 per cent) had normal coagulation times, and in an additional 6 (9.5 per cent) the coagulation time was normal on one occasion. In our own group, 8 of 11 cases had determinations which were normal on some occasions, and borderline or abnormal on others. In the other 3 cases, coagulation times were normal.

As a general rule, the coagulation time in pseudohemophilia is normal, but slight elevations, never to the degree found in hemophilia, may be present. Marked prolongation suggests either a complicating factor, such as hypoprothrombinemia, or true hemophilia †.

* Case numbers refer to listing in table 2.

† We have seen one patient not here reported, with definitely increased coagulation time (over 33 minutes), increased bleeding time, and tendency to bleed who seems to fit into some group intermediate between hemophilia and pseudohemophilia.

3 *Capillary Fragility* —Of 54 cases in which the results of a capillary fragility test (usually by the tourniquet method) were reported, 23 (42.6 per cent) showed increased fragility, 26 (48.1 per cent) showed normal fragility, and in 5 cases (9.3 per cent) the fragility was normal on some occasions and increased on others. In our 11 cases, 4 showed increased fragility, 5 showed normal fragility, and in 2 cases the capillary fragility was normal on one occasion and increased on another.

4 *Clot Retractility* —Clot retractility was mentioned in 50 cases. In 43 (86 per cent) it was normal, in 6 (12 per cent) it was abnormal, in 1 case (2 per cent) the test was normal once, abnormal another time. All our cases but one showed normal clot retractility.

Normal retraction of the clot may be considered characteristic of pseudohemophilia. The measurement of clot retractility is often carried out in a haphazard manner, which may account for some abnormal and varying results. It is known, for example, that normal clot retractility may be overlooked in a few cases if blood is allowed to stand in a glass tube for a period of time, examined, and then discarded as showing no retraction. In these cases, insertion of a glass rod or wire will cause prompt retraction about the wire, whereas for some reason retraction from the glass walls of the tube does not occur. A standardized procedure for carrying out the test should reduce the number of false negative results.

5 *Other Determinations* —In many cases determinations were made of prothrombin time, serum ascorbic acid level, serum calcium level, and plasma fibrinogen level. On the whole, values were consistently normal. In a few cases, abnormal values for prothrombin or ascorbic acid were obtained (cases 54, 56), but there was no effect on the hemorrhagic tendency when specific therapy was used.

Treatment

Calcium, vitamin K, and ascorbic acid were used to treat various cases without any effect on the bleeding tendency (cases 53, 54, 55, 56). Transfusions were usually without effect, but occasionally a good result was reported (no. 30). In the only case of our series in which transfusions were used (S. K.), two transfusions were without effect on the bleeding. Irradiation over the spleen was reported helpful in 1 case (no. 50). Splenectomy was performed in 4 cases (nos. 7, 13, 14, 30). One patient (no. 14) died 9 hours after operation, death was apparently due to continued oozing into the abdominal cavity, as autopsy revealed free intra-abdominal blood despite adequate ligation of the splenic pedicle and other blood vessels. In cases 7 and 30, no improvement followed operation. In case 13, some clinical improvement was noted, but there was no change in the bleeding time.

V THE DIAGNOSIS OF PSEUDOHEMOPHILIA

For many years it was customary to designate any bleeding disease which was not hemophilia by the term pseudohemophilia. At various times this term was applied to idiopathic thrombocytopenic purpura, fibrinogenopenia, the bleeding of leukemia, bleeding in jaundice, and to other hemorrhagic tendencies. It is only in recent years, with the advent of newer concepts of the process of coagulation and the causes of excessive hemorrhage, that study and classification have allowed the

fairly exact separation of various hemorrhagic entities. That there is a hemorrhagic disorder characterized by a tendency to bleed in the presence of normal platelets, a normal coagulation time, and an increased bleeding time, seems evident from the data presented in this report. It is to this group of cases that the term *pseudohemophilia* should be restricted. The use of this term leaves much to be desired, but it has the advantage of priority and simplicity.

Recognition of a typical case of pseudohemophilia is not difficult. Either sex is affected. The patient complains of excessive bleeding over a long period of years, often since childhood. A positive family history is frequently obtained. Physical examination is usually negative. Ecchymoses are common, but petechiae are rare. The tendency to bleed is accompanied by an increased bleeding time, an essentially normal coagulation time, a normal number of platelets, and a normal clot retraction. Differentiation from other causes of bleeding is necessary (table 4).

TABLE 4—*Differential Diagnosis of Pseudohemophilia*

	<i>Pseudohemophilia</i>	<i>Hemophilia</i>	<i>Idiopathic thrombocytopenic purpura</i>	<i>Vascular Purpura</i>
Heredity	Dominant sex linked	Recessive sex linked	None	None
Sex	M and F	M only	M and F	M and F
Transmission	Through M and F	Through F only	None	None
Petechiae	Rare	Absent	Common	Rare
Hemarthrosis	Occasional	Common	Absent	Occasional
Platelets	Normal	Normal	Decreased	Normal
Bleeding time	Increased	Normal	Increased	Normal
Coagulation time	Normal	Increased	Normal	Normal
Clot retractility	Usually normal	Normal	Markedly abnormal	Normal
Tourniquet test	Positive 50% cases	Negative	Positive	Positive

1 *Idiopathic thrombocytopenic purpura*—In this disorder a tendency to bleed is accompanied by a marked reduction in the number of platelets. The disease is rarely familial. Either sex may be affected, females somewhat more often than males. Bleeding is more or less chronic in nature, but may be acute. Physical examination characteristically reveals petechiae and ecchymoses, but is otherwise negative. The bleeding time is increased, the coagulation time normal, the platelet count definitely reduced, the clot retraction poor, and the tourniquet test positive. A typical bone marrow picture, with an increase in megakaryocytes but with greatly diminished platelet production, has recently been described.²¹

2 *Hemophilia*—In this disease a tendency to bleed is accompanied by a markedly prolonged coagulation time, with no abnormality of the other hemorrhagic tests. There is a strong familial history, males alone being affected, but only females transmitting the disorder. Bleeding is chronic in nature, usually since childhood. Physical examination reveals no abnormalities except for the evidences of bleeding. The bleeding time is normal, coagulation time markedly increased, platelet number normal, clot retraction normal, tourniquet test negative. Quick²² has recently

described a test which he considers pathognomonic for hemophilia. Tocantins^{28a} has utilized lusteroid tubes in the diagnosis of borderline cases.

3 *Fibrinogen defects*—In these extraordinarily rare disorders, a tendency to bleed is combined with a great diminution in the blood fibrinogen and a delayed coagulability of the blood. Both males and females have been reported with this disorder. The bleeding is chronic. Physical examination, except for evidences of bleeding, is negative. Laboratory examinations reveal a markedly increased coagulation time or (in cases of afibrinogenemia) incoagulable blood. The bleeding time is increased, the number of platelets is normal, and the clot retraction is poor. There is marked diminution or total absence of serum fibrinogen.

4 In *hypoprotrombinemia*, bleeding is accompanied by a reduction in plasma prothrombin (increased prothrombin time). Such bleeding is found in obstructive jaundice and in certain cases of liver disease with jaundice, and often responds to appropriate therapy with vitamin K.

5 In *leukemias* and certain other proliferative disorders, a tendency to bleed is frequently encountered. Characteristically, this tendency is due to a reduction in platelets caused by the underlying diseases, and is therefore attributable to a secondary thrombocytopenic purpura. The presence of the underlying disorder is usually detected by physical examination and appropriate hematologic studies.

6 *Vascular or nonthrombocytopenic purpura*—There is a large group of individuals who complain of recurrent ecchymoses which occur with trauma. No hematologic disorder is found and the diagnosis of vascular or nonthrombocytopenic form of purpura must be made. Whereas in pseudohemophilia, bleeding generally occurs in relation to trauma or operative procedures and ecchymoses are usually not the prominent feature, the latter are the outstanding complaint in the various forms of vascular purpura. The tourniquet test is usually positive in the latter condition and the bleeding time normal, whereas the reverse is true in pseudohemophilia. It must be conceded, however, that in certain instances it is difficult if not impossible to make a rigorous distinction between the two conditions.

VI. PATHOGENESIS

The etiology and pathogenesis of pseudohemophilia are not known. Three groups of factors are known to be concerned in the hemorrhagic disorders: plasma factors, platelet factors, and vascular factors. Each of these has been studied in cases of pseudohemophilia.

Plasma Factors

Attempts to incriminate a disturbance in pseudohemophilia analogous to that seen in hypoprotrombinemia, fibrinogenopenia, and hemophilia have thus far been unsuccessful. Thus, the blood calcium is normal, and normal plasma ascorbic acid determinations have almost always been obtained. The values for plasma fibrinogen and for prothrombin are normal. In an occasional case³³ an abnormal result for the latter determination has been reported, but administration of vitamin K with restoration of the plasma prothrombin time to normal did not eliminate the tendency to bleed.

The plasma or defibrinated serum in 3 of our cases of pseudohemophilia was

added to normal and hemophilic blood to determine whether it contained an anti-coagulant factor. In all instances, the coagulation times were reduced in a degree parallel to that exerted by normal plasma and serum. Several observers have shown that hemophilic plasma, on the other hand, contains a factor which prolongs the coagulation time of normal blood^{38a}

Quick³⁸ has recently described a test in which the recalcified plasma coagulation time is determined at varying speeds of centrifugation of the plasma. He states that the results of the test may be used for definite diagnosis in a questionable example of hemophilia. In 3 patients with pseudohemophilia, the test gave normal values.

Platelet Factors

That pseudohemophilia is not a platelet disease seems evident from the normal results obtained with all the known platelet tests. The number of platelets in the circulating blood is usually normal or high. In the few cases of pseudohemophilia in the literature in which low platelet counts were recorded, there was no correlation between the tendency to bleed and the level of the platelet count (table 2, cases no. 1, 24, 25, 32, 36, 40). In our own experience, the morphology of the platelets has never been abnormal, although some workers³³ have described vague abnormalities, as a result of which they have termed the disorder thrombasthenia or thrombopathy. In this regard, Jürgens¹⁹ devised a method for determining thrombosing-time, which he considered an index of the quality of the platelets. The exact nature of the thrombosing-time is not clear, and the difference between this measurement and that of the coagulation time in paraffin-lined or plastic tubes is questionable. Jürgens claimed that the thrombosing time was increased in cases of pseudohemophilia, and that therefore the basic fault in this syndrome was some qualitative difficulty in the platelets which prevented their agglutination in normal time.

Clot retractility is to a large degree a measurement of the quality and number of platelets. Normal clot retractility is characteristic of pseudohemophilia, another indication of the normality of the platelets in this disorder.

Vascular Factors

1 *Capillary Fragility*—The tourniquet test is positive in about one-half of the cases of pseudohemophilia. This finding immediately suggests (in the absence of thrombocytopenia) a vascular defect. The fact, however, that the test is positive in only one-half of the cases suggests that capillary fragility is not the only factor involved in the disorder. It is of interest that certain cases which show a normal capillary fragility on some occasions present abnormal results on others, perhaps suggesting that some change in the capillaries is responsible in part at least for the excessive bleeding.

2 *Petechiae*—Although petechiae are regularly present and numerous in thrombocytopenic states, they also occur in nonthrombocytopenic purpuras. The rarity of petechiae in pseudohemophilia, and their transient nature, are perhaps indirect evidence of their occurrence on a vascular rather than on a platelet basis.

3 *Capillary Visualization*—MacFarlane³⁵ demonstrated a capillary defect in

pseudohemophilic patients by means of direct visualization of the capillaries in the nail bed, and by noting a difference in reaction to trauma in pseudohemophilic and normal individuals. He found that the capillaries of pseudohemophilics were distorted and bizarre in appearance and failed to contract promptly after puncture. Normal capillaries, in contrast, contracted quickly following puncture. MacFarlane accordingly attributed the bleeding of pseudohemophilia to a vascular (capillary) defect, in which a disturbance in contractility was the outstanding abnormality. The capillaries of the nail beds were studied in 2 of our cases (S. K. and F. W.). In both instances, normal findings were obtained. Puncture studies, however, were not attempted.

Role of Heredity

Many cases of pseudohemophilia show a positive family history. This was best demonstrated in our series by case F. W., in whose family 16 of 42 individuals in four generations showed a tendency to bleed. Transmission in these cases may be through either sex, and in direct line from one generation to the next. Von Willebrand's studies in this regard are the most comprehensive, and, according to him, the disease is due to a dominant sex-linked gene. Inbreeding may have been a factor in some of von Willebrand's Finnish families, who lived on a small isolated island. Since most of our cases failed to demonstrate a familial history, some of von Willebrand's conclusions are open to question although undoubtedly they were correct for his own particular series.

In summary, most data tend to classify pseudohemophilia as a disorder of the vascular apparatus. The lack of evidence for a plasma or a platelet disturbance, and the existence of some direct evidence for vascular abnormalities, suggest that pseudohemophilia is a form of nonthrombocytopenic vascular purpura, in which a hitherto undemonstrated defect in the capillary system is present.

VII. THERAPY AND PROGNOSIS

The treatment of pseudohemophilia is unsatisfactory. Numerous therapeutic methods have been utilized, usually without effect on the bleeding tendency. Calcium in large doses, vitamin K, and ascorbic acid have been completely without effect. Transfusions showed no effect on the bleeding. Splenectomy has been performed without benefit. The only bleeding disease in which removal of the spleen is of proven value is idiopathic thrombocytopenic purpura. Here the bleeding can be correlated with a reduction in the number of platelets, and splenectomy is followed by a quick rise in this factor. In pseudohemophilia, the number of platelets is normal, and the bleeding tendency is not correlated with them. There is thus no evidence that the spleen is in any way concerned with the disease. Splenectomy furthermore carries the usual risk of any operation in these individuals.

Forms of treatment must therefore be largely prophylactic. A patient with a history of easy bruising or other tendency to bleed should be investigated with reference to the possible existence of a hemorrhagic diathesis. If he fits into the category here described, operations should be avoided if possible. In one of our

cases (R C) it was necessary to abandon an operation because of uncontrollable bleeding after incision. Necessary operations may be undertaken with proper aids, such as thrombin and fibrin foam, and the knowledge that hemorrhage may be severe enough to require transfusions.

In cases of active hemorrhage, the only reliable measures are pressure and the use of locally applied thrombin-fibrin foam.* These measures are especially feasible in hemorrhage from the nose and from tooth sockets. A fibrin pad is soaked in thrombin foam to form a gelatinous mass, which is then applied over the area of hemorrhage. Bleeding often ceases promptly. Direct pressure packing with either plain or thrombin soaked gauze may also stop bleeding promptly. Transfusion is given as indicated for post-hemorrhagic anemia.

In certain cases with brisk bleeding, as from the kidneys or the uterus, the treatment may be far more difficult than in hemophilia or idiopathic thrombocytopenic purpura. In the former, a transfusion or two or the use of the recently fractionated antihemophilic plasma globulin²⁹ will usually shorten the coagulation time and control the bleeding. In idiopathic thrombocytopenic purpura, cessation of active bleeding and complete cure in most cases is effected by splenectomy. In pseudo hemophilia, on the other hand, where complete normality of both plasma and platelet factors is present, brisk bleeding may be difficult and even impossible to control. Thus, transfusion was without effect in several episodes of bleeding in case S K, and splenectomy was ineffective (and at times even fatal) in some of the cases reported in the literature.

Death in pseudo hemophilia is rarely due to bleeding. Only 2 cases in the literature (table 2, nos 14 and 20) died of excessive hemorrhage, although the familial history in proven cases often included poorly studied bleeders who died of blood loss.²³ The prognosis for life is excellent. The tendency to bleed, however, remains essentially unchanged throughout life, although in some instances the frequency of bleeding (especially epistaxes) decreases after childhood. Knowledge of the existence of the disorder in a given individual may be expected to prevent serious consequences by prompt local treatment at sites of bleeding and by avoidance of all non essential surgical procedures.

SUMMARY

1 Eleven cases of a hemorrhagic diathesis are presented characterized by an increased bleeding time in the presence of a normal coagulation time, normal blood platelet count, and normal clot retraction. An analysis is made of 62 similar cases in the literature.

2 These cases, which have been designated as pseudo hemophilia, probably represent a particular disorder of the capillaries, in which capillary retractility following trauma may be inherently defective.

3 The differential diagnosis of these cases from other types of hemorrhagic disease and the necessity for their recognition particularly from the prophylactic standpoint are stressed. Except for easily accessible local bleeding, therapy is at present ineffectual.

* The synthetic fibrin and the bovine thrombin of Upjohn Co. have been used in our studies.

4. Standardized methods for the performance of hemorrhagic tests are suggested. The Lee White method for the coagulation time may give normal values as high as 20 minutes or more.

APPENDIX ON METHODS

In the literature relating to the hemorrhagic diseases, the following tests have been most commonly used: coagulation time, bleeding time, clot retractility, platelet count, and tourniquet test. These tests are analyzed below, and are suggested as standard procedures whose duplication would allow comparison of results from various laboratories.

Coagulation Time

1. *Technics*.—(a) *Lee-White method*¹⁰ One cc. of blood is withdrawn from a vein using a sterile syringe rinsed with normal salt solution, and transferred to a glass test tube with inside diameter of 8 mm. previously rinsed with normal salt solution. In transferring the blood to the tube the barrel of the syringe is pushed very gently, so that the blood flows slowly and foam is thereby avoided. A stopwatch is started as soon as the blood appears in the syringe. The test tube is allowed to stand at room temperature and inverted every 30 seconds. The end point is the time at which the blood no longer flows on inversion of the tube. Normal values, according to Lee and White, are 5 to 8 minutes.

(b) *Lee White method in three tubes*. With a sterile dry syringe and a sterile 19 to 21 gauge needle blood is drawn from a median antecubital vein and 1 cc. is placed into each of three dry clean glass test tubes with inside diameter of 8 mm. A stopwatch is started as soon as blood appears in the syringe. The first tube is tilted every 30 seconds until a clot is formed sufficient to prevent any flow of blood when the tube is inverted, this is listed as the first end point. After the first tube has clotted in this way the second tube is similarly handled until its end point is reached. After coagulation has occurred in the second tube the end point of the third tube is similarly determined. The result is therefore expressed as three separate numbers, e.g., 5, 7, 10 minutes. Normal is usually given as 5 to 10 minutes.

(c) *Lee White method at 37° C.*¹¹ One cc. of blood obtained as above is placed in a clean dry glass tube with inside diameter of 8 mm., which is then placed in a water bath maintained at a temperature of 37° C. A stopwatch is used as above to time the interval between the appearance of blood in the syringe and the formation of a clot in the test tube sufficient to prevent flow of blood on inversion of the tube. Normal is given as 5 to 8 minutes.

2. *Results*.—Table 5 lists representative results of coagulation time determinations performed by these methods in normal individuals. Two facts stand out: (1) The range of normal values by the three tube method at room temperature is approximately 10-14 16½ minutes, which is definitely higher than the normal figures, and the average value in the first of these tubes which represents Lee and White's original technic, is also higher than their results. (2) The use of a single tube at 37° C. gives a normal range of 7½ to 15 minutes again much higher than the so-called normal values of 5 to 8 minutes.

3. *Critique*.—In our laboratory we usually use the modified Lee-White method with three tubes described above as method (b). Apparently many different technics are employed under the designation of Lee White, each laboratory using its individual modification for performing the determination. The only points of unanimity are the use of venous blood and of glass tubes.

Lee and White gave normal values of 5 to 8 minutes for the determination with an average of 6½ minutes. Various workers have modified the original technic, usually by using anywhere from two to six tubes and give other values for normal. Thus, Wintrobe gives 6 to 15 minutes¹², Downey about 12 minutes¹³, Kracke, between 5 and 10 minutes¹⁴, Quick¹⁵ and others^{16, 18} 5 to 8 minutes. Lenoble, 2 to 25 minutes¹⁹.

Part of the reason for such variations lies in the many possible variables in technic, including variations in temperature, wettability of the test tube, diameter of the test tube and frequency of tilting.^{19, 22} It is obviously difficult, therefore, to compare the results obtained in one laboratory under a particular set of experimental conditions with those obtained in another laboratory under different conditions, although both are called the Lee White method. As a designation for a particular determination of the coagulation time this term has become meaningless.

In reviewing the literature therefore, it is necessary to make liberal allowances for these facts, and we have considered normal to be certainly as high as 16 minutes and probably (in some instances) up to 20 minutes, by the usual method of venous blood in glass tubes. This method has been used by most writers, a few employ the capillary tube method, in which the normal value is about 3 minutes. In the future, we plan to use one glass tube at 37° C. in order to avoid variations in room temperature. Normal values are about 10 minutes.

TABLE 5—Coagulation Time Determinations in Representative Normal Controls

Control	Room temp			37° C
	Tube 1	2	3	
1	13'	19'		
2	12'	12'30"	13'30"	
3	14'30"	17'30"	20'30"	
4	13'30"	15'	22'30"	
5	9'30"	11'	14'30"	
6	5'	5'	5'	
7	8'	10'	15'	
8	6'	12'	12'	
9	10'	13'30"	16'	
10	4'30"	12'	12'30"	
11	11'	16'30"	18'	
12	14'30"	19'	19'	
13	11'	13'	13'	13'
14	10'30"	15'30"	18'30"	12'
15	12'	12'	12'	8'
16	7'30"	10'30"	10'30"	7'30"
17	13'	17'	19'30"	10'
18	12'	22'	26'	9'
19	9'	15'30"	18'30"	11'30"
20	20'	20'	26'	15'
21	10'	13'	16'	9'30"
Mean	10'19"	14'21"	16'26"	10'37"
Lowest	4'30"	5'	5'	7'30"
Highest	20'	22'	26'	15'

Bleeding Time

1. *Technique*—(a) *Duke method modified*²³ The lobe of the ear is cleansed with 70 per cent ethyl alcohol and wiped dry. A clean glass slide is placed behind the ear lobe and the lobe steadied on the glass slide. With a Bard Parker blade no. 11 mounted in a cork stopper and held in the other hand, a quick thrust is made through the ear lobe hitting the glass slide. At intervals of one half minute, the drop of blood exuding from the cut is wiped away by a piece of filter paper, without touching the skin. Spontaneous cessation of the bleeding is timed with a stopwatch. The bleeding time is the time from the production of the stab-wound in the ear lobe to the cessation of bleeding. In cases of unusually severe or profuse bleeding, a piece of gauze is used (during the time of severe bleeding) to absorb excess blood, but without pressure or contact with the incised wound.

It has been our practice to perform the bleeding time test on both ears, to minimize as far as possible certain local variations. Normal by this method is 1 to 3 minutes rarely up to 5 minutes.

(b) *Ivy method*²⁴ A blood pressure cuff is applied about the arm above the elbow and inflated to 40 mm of mercury. An area on the forearm free of visible veins is cleansed with alcohol, dried and punctured.

with an automatic lancet to a depth of 3 mm. The exuding blood is wiped away every 30 seconds with filter paper as outlined under the Duke method. The time, measured on a stopwatch, between the production of the puncture and the spontaneous cessation of bleeding is taken as the bleeding time. Ninety-seven per cent of over 100 normal individuals showed a bleeding time by this method of 6 minutes or less.⁶

2. *Critique*—The significance of the bleeding time is still somewhat obscure. Its value apparently depends partly on the number of platelets in the circulating blood and partly on peripheral (vascular and tissue) factors. Disturbance in either factor may result in an increased bleeding time.

The Duke method is satisfactory for determination of bleeding time. Our modification consists in the use of a sharp no. 11 Bard Parker blade with which the ear lobe is pierced. We believe that this reduces to a minimum the admixture of tissue juices and at the same time standardizes the depth of the stroke. The Ivy method is also an attempt at standardization and is also satisfactory. In occasional cases the Ivy method shows an abnormally high bleeding time when the Duke method is normal and vice versa.

Clot Retractivity

1. *Technic*—One cc. of blood, obtained by venipuncture with a dry clean syringe and a dry sterile 19 to 21 gauge needle, is placed in a dry clean glass test tube 5 x 100 mm. in size. The tube is placed in an incubator at 37° C. and observed at 30 minutes, 1 hour, 2 hours, and 24 hours.

2. *Results*—Normally, retraction of the clot from the walls of the test tube has started by 30 minutes, is appreciable by 1 hour, and is complete by 24 hours.

In some cases retraction appears absent, but occurs promptly when a wire or glass rod is inserted into the standing blood, for some reason, the blood retracts more readily from such an inserted instrument than from the walls of the test tube. Such a result is normal. If a wire rod was used to separate the clots from the test tube, clot retraction was present within 30 minutes in 94 per cent of over 100 normal individuals. If no such precaution was taken, 30 per cent of the same subjects showed no retraction in 30 minutes.⁶⁷

3. *Critique*—Good retractility of the blood clot is generally taken to be synonymous with a normal number of platelets, and poor or absent retractility with a reduction or absence of platelets. According to Evans,⁶⁸ there is a rough but distinct parallelism between the number of platelets and the quality of clot retraction. Negatively, poor retractility is generally accepted as meaning decreased number and/or poor quality of the platelets.

This strict correlation has been questioned by a number of observers. Hayem,⁶⁹ Whitby and Britton,⁶⁸ and Mackay⁶⁹ have all commented on the poor or absent retraction of the clot in pneumonia despite the normal platelet count, and they have attributed it variously to an antifibrin factor in the plasma (Hayem), lack of fibrinogen in the blood (Whitby and Britton), or some disturbance in the blood plasma (Mackay). The intravenous injection of peptone into experimental animals is known to cause incoagulability of their blood, if, however, the amount of material injected is properly chosen. Coagulation without clot retraction can be produced although the platelet count is normal (Gley⁶⁹). Gley also produced nonretraction of animal blood by intravenous injection of heterologous blood and of diphtheria toxin, and postulated a modification of the fibrin produced during coagulation under his experimental conditions. Finally, Gordon⁶¹ noted nonretraction of the blood clot in 40 per cent of a large random series of specimens taken for routine Wassermann and agglutination tests.

It seems probable that normal clot retraction depends on at least two factors, one of which is the number of platelets present, and the other some ill-defined factor in the patient's blood or plasma. In most cases the more important factor of the two is undoubtedly the number of platelets, but it no longer seems safe to assume without further investigation that nonretractility means thrombocytopenia.

Platelet Count

1. *Technic*—(a) *Indirect method*⁶² A finger is well cleaned with alcohol or acetone and then dried. A puncture wound is made in the finger tip and the first drop of blood is discarded. A large drop (3 mm.) of staining solution* is placed over the puncture wound, and the finger gently squeezed so that a small

* Brilliant Cresyl Blue 0.15, sodium citrate 0.40, sucrose 8.00, water 100. Dissolve, filter, and add 3 drops of U.S.P. formaldehyde 1:10 to the filtered solution.

amount of blood (about one sixth of the amount of staining fluid) wells up into the drop of staining solution

A clean cover slip is then touched to the combined drop of blood and fluid, and the cover slip dropped onto a clean slide. The cover slip is ringed with paraffin, and the preparation examined after 15 to 40 minutes of standing. Platelets appear as highly refractile opalescent bodies with a pale bluish stain. Reticulocytes are also stained by this method.

Under oil immersion 1000 red blood cells are counted and the number of platelets seen during this enumeration is recorded. The total number of platelets per cubic millimeter is obtained by means of the equation

$$\frac{\text{Number platelets per 1000 RBC}}{1000 \text{ RBC}} = \frac{\text{Total platelets per cu. mm}}{\text{Red Count per cu. mm}}$$

or,

$$\text{Platelets per cu. mm} = \text{Number counted} \times \frac{\text{red count}}{1000}$$

Normal by this method is 400 000 to 900 000

(b) *Direct methods*.⁶² Various direct methods have been described for platelet determination, all depending on the use of some diluting fluid which tends to prevent agglutination of platelets. The direct methods are most frequently recorded in literature. The normal number is usually given as about 200 000 platelets per cubic millimeter, with normal values often as low as 150,000 or less, and as high as 350,000. We have used the figure 150,000 as the lower level of normality in our review of the literature.

In most work, the indirect method allows easier enumeration of the platelets and at the same time permits computation of reticulocytes. The accuracy of the count is enhanced by the fact that the oil immersion lens is used, facilitating the recognition of platelets. However, any method uniformly utilized by the same individual will give results which may properly be compared with each other. The normal value of one's method should be stated in all instances.

Capillary Fragility (Tourniquet Test)

1 *Technic*.—Four centimeters below the bend of the elbow anteriorly a circle is drawn the size of a twenty five cent piece (approximately 2.5 cm. diameter). Any petechiae present are noted. The cuff of a sphygmomanometer is placed about the arm in the usual way, and the blood pressure taken. The cuff is then inflated to a point halfway between the systolic and diastolic pressures, and this level is maintained for 5 minutes. The number of new petechiae seen with the naked eye in the inscribed circle is counted. Variations in this test have consisted chiefly in the level at which the pressure is maintained and the time of its maintenance. The standard method above is suggested for uniformity.

2 *Results*.—Of over 100 normal individuals, 89 per cent showed fewer than 10 petechiae, and an additional 7.4 per cent showed from 10 to 20 petechiae. The presence of over 10 new petechiae in the inscribed circle is taken as evidence of a positive test (i.e., increased capillary fragility). Preliminary observations suggest that possibly if the pressure time is 3 minutes instead of 5 minutes, fewer normal individuals will show over 10 petechiae.⁶⁷

3 *Critique*.—The test is positive in both increased capillary permeability and thrombocytopenic conditions.

Other Tests

1 *Prothrombin Time (Quick)*.⁶⁸—Normal is about 12 to 17 seconds. A normal control is run as a check.

2 *Ascorbic Acid Determination*.⁶⁴—Normal is 0.4 to 1.0 mg. per 100 cc. plasma.

3 *Plasma Fibrinogen*.⁶⁸—Normal is 0.25 to 0.5 grams per 100 cc. blood. Blood which lacks fibrinogen will not clot, blood poor in fibrinogen will clot very slowly. If the clotting time is normal or only slightly delayed, fibrinopenia and afibrinogenemia need not be considered as possibilities.

4 *Serum Calcium*.⁶⁴—Normal is 9.0 to 10.5 grams per 100 cc. blood. Hemorrhagic disease due to hypocalcemia has not been described.

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ON THE INFLUENCE OF STILBAMIDINE UPON MYELOMA CELLS

By I SNAPPER, M D , AND B SCHNEID, M D

ONE of us¹ has reported that in certain cases of multiple myeloma, considerable improvement of the clinical condition can be obtained by intravenous or intramuscular injections of stilbamidine (diamidino-stilbene di-isethionate)* It is necessary that the patient follow a diet low in animal protein A favorable result has been observed in 9 out of 10 patients During this treatment with stilbamidine, histological changes can be observed developing in the myeloma cells

The basophilic cytoplasm of myeloma cells is usually vacuolated and, on the whole, is free of granules or other inclusions From time to time a few azurophilic granules and tiny azurophilic rods, resembling Auer bodies, are found Exceptionally these azurophilic inclusions grow to a larger size

During a course of injections of stilbamidine large basophilic granules appear in the cytoplasm of the myeloma cells which are without difficulty visualized in bone marrow smears by Wright or Giemsa stains These granules have first been observed after the treatment has lasted 3 to 4 weeks The amount of stilbamidine administered up to the time when the granules appear has varied between 1875 and 3600 mg In the beginning the granules have a faint resemblance to swollen cocci When the treatment is continued the granules become confluent, so that after several weeks of treatment large deeply blue precipitates, resembling inclusion bodies, are found in the cytoplasm of most myeloma cells (fig 1)

Supravital staining of the myeloma cells of untreated myeloma cases with Janus green and neutral red reveals the presence of a considerable number of green staining mitochondria In the myeloma cells of myeloma cases treated with stilbamidine the cytoplasm contains not only green mitochondria but also large red globules Although it cannot be proved that these red granules visualized by the vital stain are identical with the blue granules found in the Wright stain, it may be stressed that both types of granules develop at the same time

The cytoplasm of myeloma cells stains deeply red with pyronine if Unna's phenol methylgreen pyronine stain is used In the cytoplasm of the untreated myeloma cases this stain does not reveal the presence of any granules In the patients in whom stilbamidine treatment has given rise to the development of the granules mentioned above, the Unna stain shows the presence of bright red granules in the red cytoplasm

The granules which develop in the myeloma cells under influence of the stilbamidine treatment are not metachromatic because they do not stain with Unna's polychrome methylene blue The granules do not show positive oxydase reactions

In 7 out of 9 cases of multiple myeloma treated with stilbamidine, these inclusion bodies were consistently present and could be found in 80 to 90 per cent of the myeloma cells of the bone marrow In none of the other bone marrow elements

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* Manufactured by May and Baker, Dagenham, London

could comparable granules be demonstrated. In one of these 7 patients myeloma cells could be found in the peripheral blood. During the stilbamidine treatment these cells also presented the basophilic granules mentioned.

It is easy to distinguish these basophilic bodies from the large azurophilic inclusions which exceptionally are found in the myeloma cells of untreated myeloma patients. The azurophilic inclusions always appear reddish blue in Wright or Giemsa preparations. They do not take the pyronine stain, and they cannot be visualized by neutral red in the vitally stained preparations.

In highly interesting *in vitro* experiments, Kopac² demonstrated that stilbamidine dissociates protamine ribonucleate and releases the protamines from these compounds. This mechanism may well be responsible for the changes visualized in the

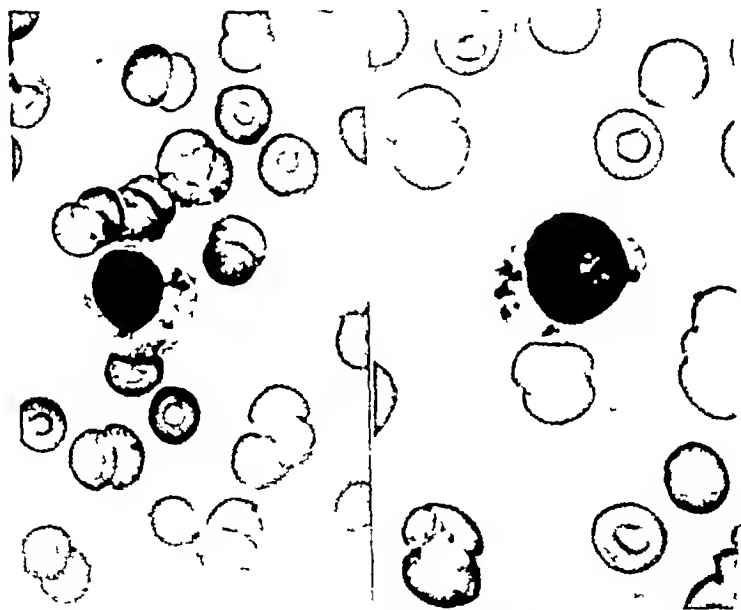


FIG. 1. BONE MARROW SMEAR OF A PATIENT WITH MYELOMA TREATED WITH STILBAMIDINE.

Myeloma cells contain basophilic inclusions which are stained deeply blue with Wright stain.

myeloma cells under influence of stilbamidine. Evidence available indicates that ribose nucleic acid forms one of the main constituents of the basophilic inclusion bodies, described above.*

SUMMARY

Injections of stilbamidine cause morphological changes in myeloma cells. During this treatment large basophilic granules appear in the cytoplasm which show a tendency to become confluent. These granules stain red with pyronine and can be

* As far as we can see the myeloma cells containing inclusion bodies do not deteriorate ultimately. However, in view of the fact that these bodies represent precipitates of ribose nucleic acid the metabolism of the cell must have been thrown out of gear. Personally, we have the feeling that only the extension of the myeloma areas is inhibited. This would explain the cessation of pains. An actual cure, at least for the time being, we have not observed.

visualized in the supravital stain with neutral red. One of the main constituents of these inclusions consists of ribose nucleic acid.

These morphological changes seem to be limited to myeloma cells, since in none of the other bone marrow elements do comparable granules or inclusions develop.

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HEMATOLOGICAL CHANGES FOLLOWING THE ADMINISTRATION OF LARGE DOSES OF QUINACRINE HYDROCHLORIDE

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THERE is a paucity of data in the literature on blood morphology following the administration of quinacrine hydrochloride (atabrin dihydrochloride) Hecht¹ observed no formation of methemoglobin *in vitro* or *in vivo* and no hemolytic action He reports that 1 rabbit which received 100 mg per Kg for 6 successive days showed no change in erythrocyte count or hemoglobin content of the blood In a group of 20 Europeans living in Liberia, each of whom received 143 Gm of quinacrine over a period of 7 years (average of about 0.05 Gm daily), Junge² found no blood changes Piorkowski³ reported on a patient in whom agranulocytosis appeared after the administration of quinacrine, but since several other drugs, including a sulfonamide, were also given, this blood change cannot be attributed to atabrin alone Recently Barlow et al.⁴ stated that after prolonged oral administration of quinacrine to rats in doses up to 180 mg per Kg they found no significant effect on the blood Fitzhugh and co-workers⁵ fed the drug to rats in the diet and observed basophilic inclusions in the lymphocytes and a leukocytosis, thus confirming our earlier hematological findings^{6,7}

In view of the increased use of quinacrine, it was considered desirable to investigate the effects of atabrin on the blood morphology with the hope that such a study might reveal criteria which may be used as indicators of toxicity The present communication is an extension and completion of our preliminary study⁷

MATERIALS AND METHODS

Nine species of laboratory animals were used in these experiments These included 180 rats, 45 mice, 13 albino rabbits, 16 guinea-pigs, 17 hamsters, 13 mongrel dogs, 11 monkeys (*Macacus rhesus*), 6 ducks (white Pekin), and 28 chickens (single comb white leghorn) The usual stock rations were fed to the animals during the investigation The avian species were housed first in group cages or large bins at a temperature of 80° F, later at room temperature The mammalian species were kept in an air-conditioned laboratory at a temperature of 75° F and relative humidity of 50 per cent, the mice in small group cages, the others in individual cages

Aqueous solutions of quinacrine were freshly prepared and administered orally by metal catheter and syringe or by stomach tube, depending upon the size of the animal The dose levels selected could not be based on chemotherapeutic efficacy, since the duck, chicken, and monkey are the only species used which can be infected with malaria For this reason, the levels administered were based on the

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dose (L D 50 for the rat) which kills 50 per cent of a group of rats within 24 hours. This value, in a series of 100 rats, was found to be 900 mg per Kg. Although this mortality figure does not hold for species other than the rat, it nevertheless gives an indication of the dose range which might produce similar effects.

The rats, mice, rabbits, guinea-pigs, hamsters, chickens, and ducks were dosed daily, and the dogs and monkeys six times weekly. One monkey receiving 300 mg per Kg was dosed only three times per week. The dose levels of quinacrine administered to the various species are shown in tables 1 and 2.

Standardized blood pipets were used for all counts. Erythrocyte counts were made with Hayem's solution and leukocyte counts with 2 per cent acetic acid, tinted with gentian violet as the diluent. The diluting fluid of Hirschfeld and Hortink⁸ was used for blood counts in the chickens and ducks. Duplicate counts were made initially on all animals and subsequently when a deviation from the

TABLE 1—Minimum Time (Days) Required for Production of Blood Changes in Rats after Quinacrine Treatment

No of rats	Dose	Dose % of L.D. 50	Range of deaths	Anemia			Leukocytosis	Polynucleosis	Lymphopenia	Monocytosis	Lymphocytic inclusions	Erythrocytic inclusions
				Slight	Moderate	Marked						
	mg. per Kg.		days									
10	225	25	5-8	neg	neg	neg	neg	2-3	2-3	neg	3	7
20	90	10	11-38	14	27	35	18	18	18	18	4	10
20	45	5	23-93	28	35	63	21	21	21	18	14	28
20	18	2	none*		neg		119	119	119	119	30	neg
20	9	1	none*		neg		neg	neg	neg	neg	neg	neg
20	4.5	0.5	none*		neg		neg	neg	neg	neg	neg	neg
20	0.9	0.1	none*		neg		neg	neg	neg	neg	neg	neg
20	Controls		none*		neg		neg	neg	neg	neg	neg	neg

* All animals living at 4 months

normal was apparent. Differential counts were made on Wright's stained smears. Hemoglobin determinations were carried out in a Sahli-Hellige hemoglobinometer. Erythrocyte, leukocyte, and differential counts were made in all species but the mouse. In this species differential counts alone were made. In dogs, sedimentation rate, hematocrit, and icteric index readings were done, using the Wintrobe hematocrit tube with a potassium oxalate-ammonium oxalate mixture as anticoagulant. Prothrombin values in rats were obtained by an adaptation of Hoffman and Custer's micro-method,⁹ and in dogs by the macro-method of Campbell et al.¹⁰

OBSERVATIONS

Rats. The average weight of the rats used was 193 grams. The rats receiving 225 mg per Kg (25 per cent L D 50) daily died within 8 days. Within 3 days an increase in the size and number of vacuoles in the cytoplasm of the lymphocytes was noted. Dark blue staining bodies appeared in the cytoplasm of these cells.

TABLE 2.—*Incidence of Lymphocytic and Erythrocytic Inclusions after the Administration of Quinacrine*

Species	Average weight	Number of animals	Dose <i>mg per Kg</i>	Lymphocytic inclusions	Erythrocytic inclusions
1 Mouse	20 Gm	10	225	+	+
		5	90	—	—
		10	45	—	—
		10	45	—	—
		10	0	—	—
2 Rabbit	2.8 Kg	3	90	+	—
		4	45	+	—
		3	0	—	—
3 Guinea pig	750 Gm	2	225	+	—
		6	90	+	—
		4	45	—	—
		4	0	—	—
4 Hamster	102 Gm	2	900	+	—
		4	225	+	—
		5	90	+	+
		3	45	—	—
		3	0	—	—
5 Dog	8.6 Kg	3	50	+	—
		1	50-5	—	—
		5	30	—	—
		4	0	—	—
6 Monkey	3.1 Kg	1	300	+	—
		1	200	+	—
		1	100	+	—
		1	40	—	—
		1	20	—	—
		3	10	—	—
		3	0	—	—
7 Chicken	60 Gm	4	900	+	—
		4	225	+	—
		5	90	+	—
		5	45	—	—
		5	9	—	—
		5	0	—	—
8 Duck	80 Gm	2	90	+	—
		2	45	+	—
		2	0	—	—

(fig 1) The inclusions varied in size and shape from minute punctate granules to large irregularly shaped masses which sometimes caused a bulging of the cell. These inclusions often appeared to partially occupy the vacuolar areas. Vacuoles

and inclusions sometimes appeared in lymphocytes independent of each other. However, the cells containing inclusions usually showed some degree of vacuolation. In some animals 100 per cent of the lymphocytes eventually contained inclusions. So distinct is the morphology and especially the staining qualities of the inclusions that they stand out clearly when present with azurophilic granules in the same cell. Within 2 or 3 days the rats in this group also developed a lymphopenia and polynucleosis.

After 1 week, small irregularly shaped vacuoles, accompanied in most instances by a dark blue staining material which resembled that found in the cytoplasm of the lymphocytes, appeared in many of the red blood cells. The blue inclusions in



FIG. 1. LYMPHOCYTE OF RAT GIVEN 5 DAILY DOSES OF QUINACRINE (22.5 MG PER KG). Basophilic inclusions and vacuoles present in cytoplasm. Wright's stain. 2300 X.

the erythrocytes seemed at times partially to fill the vacuoles, just as was noted in the lymphocytes. Polychromatophilic cells were always affected to a greater extent than erythrocytes, which took a normal stain. The lymphocytic and erythrocytic inclusions could be stained with Giemsa's and Leishman's stain as well as with Wright's solution.

At the 90 mg per Kg level (10 per cent L.D. 50) the rats died in 11 to 38 days. Within 2 weeks a slight anemia was evident in most rats. This progressed to a moderate anemia in rats surviving for longer periods, and those rats which lived for 5 weeks showed a marked anemia (fig. 2). The lymphocytic inclusions appeared within 1 week and increased in number until an average of about 50 per cent of the cells was affected. The average percentage of lymphocytes with inclusions then remained relatively constant until the animals died. An occasional monocyte

showing inclusions similar to those found in the lymphocytes was found in those rats showing a marked monocytosis. In $1\frac{1}{2}$ to 2 weeks, erythrocytic inclusions and vacuoles were evident. After $2\frac{1}{2}$ weeks many rats showed a leukocytosis accompanied by a relative or absolute lymphopenia, polynucleosis, and monocytosis. Additional dosing resulted in further increases in the leukocyte counts. Absolute lymphopenia, polynucleosis, and monocytosis were then evident. Figure 3 shows the trend of the differential counts in these rats.

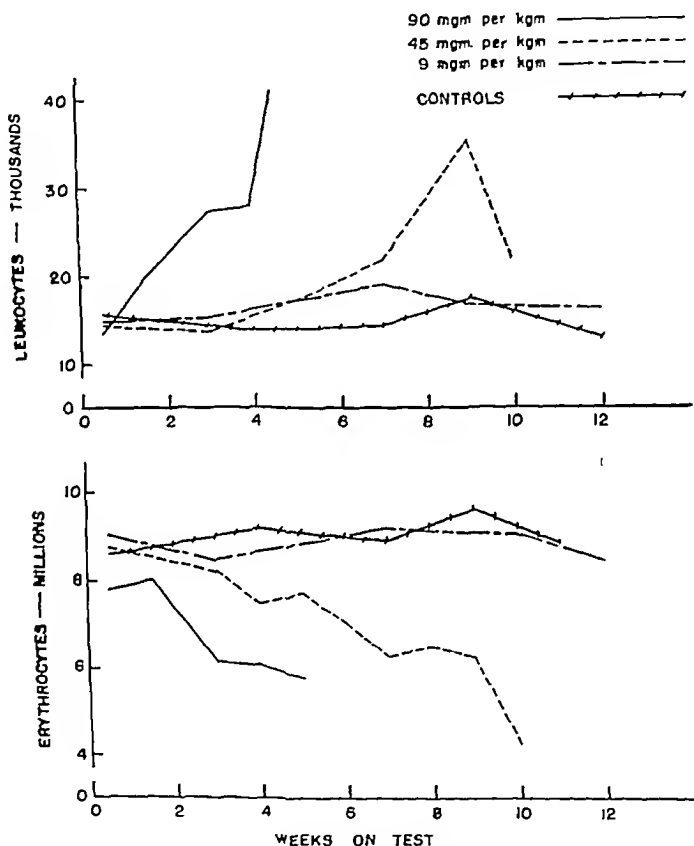


FIG. 2. BLOOD COUNTS IN RATS DOSED ORALLY WITH QUINACRINE
Each curve represents the average counts in 10 animals

Since in rats hepatic necrosis results from quinacrine administration,⁶ it seemed of interest to study the prothrombin levels of animals thus affected. By abdominal palpation of rats dosed with 90 mg per Kg a somewhat enlarged and firm liver could be detected. Determinations of prothrombin levels on 15 rats suspected of having liver necrosis as judged by palpation gave normal values. The presence of liver necrosis was confirmed in these animals after death.

The rats receiving 45 mg per Kg (5 per cent L D 50) died in 23 to 93 days. A slight anemia became evident in this group at 4 weeks, was moderate in 6 to

8 weeks, and became very marked in several animals in 9 weeks (fig. 2). The red cell count of 1 animal decreased from 9.29 to 2.15 million and the hemoglobin from 14.5 to 4.0 grams. The lymphocytic inclusions were observed in 2 weeks and the erythrocytic inclusions in 4 weeks. About this time there was a progressive increase in the leukocyte count in most rats, accompanied by changes in differential count similar to those noted in the 10 per cent L D 50 group. A slight monocytosis was first noted after 2½ weeks, and in 5 to 7 weeks the rats had an absolute lymphopenia, polynucleosis, and monocytosis. Some rats died shortly after showing their highest white cell counts, while in others a peak was followed by a decline. The latter animals died, however, before the counts had returned to normal. The abnormal differential counts were most marked at the height of leukocytosis.

The rats receiving 18 mg. per Kg. (2 per cent L D 50) or less, as well as the controls, maintained normal red blood counts and hemoglobin values. After 4 months no erythrocytic inclusions were found, but the test group showed lymphocytic inclusions within 1 month. An occasional rat receiving 2 per cent or 1 per cent L D 50 revealed a slight leukocytosis accompanied by a slight lymphopenia, polynucleosis, and monocytosis, but those receiving less than 1 per cent L D 50 as well as the controls maintained normal blood values. Table 2 shows the time required for blood changes in all groups of rats.

Since the lymphocytic and erythrocytic inclusions were found only in rats dosed with quinacrine and never in the controls, the question arose as to whether these inclusions were due to a specific action of the drug or were due to a secondary effect such as the reactivation of a latent *Bartonella* or similar infection. To test this second possibility a group of 22 rats averaging 225 grams in weight was dosed daily with 90 mg. of quinacrine per Kg. (10 per cent L D 50) for either 4 or 9 days only. Blood smears were made at frequent intervals during and after the dosing period and examined for inclusions in the lymphocytes and red blood cells. After 4 doses of quinacrine an average of 25 per cent of the lymphocytes of these rats contained inclusions. The percentage of lymphocytes with inclusions continued to increase in all animals and reached a peak within 9 days, irrespective of the number of doses of quinacrine given. However, the percentage of lymphocytes involved was slightly greater in the animals receiving the greater number of doses. After the peak level was reached the percentage of lymphocytes containing inclusions decreased at approximately the same rate in all animals until none could be found after about 1 month (fig. 4). Erythrocytic inclusions were observed in small numbers in rats which received 9 doses of quinacrine. These inclusions were absent from the red blood cells after 1 month.

To study further the relationship of a possible infection to the lymphocytic and erythrocytic inclusions, an attempt was made to induce these inclusions in rats and rabbits by blood transfusion. Heparinized blood, from rats in which 50 per cent of the lymphocytes and a large number of erythrocytes contained inclusions, was injected intravenously into 5 normal weanling rats (0.2 cc. each), 3 splenectomized adult rats (0.3 cc.) and 3 young rabbits (0.5 cc.). Blood smears were then made from the recipients at 3 day intervals for 1 month and examined for inclusions. All smears were found to be negative.

Mice Mice receiving 225 mg per Kg died within 2 to 11 days. Lymphocytic inclusions were seen within 6 days in 8 of 10 mice. An average of 14 per cent of the lymphocytes contained inclusions on the ninth day and 20 per cent were affected by the twentieth day. Erythrocytic inclusions appeared in all animals within a

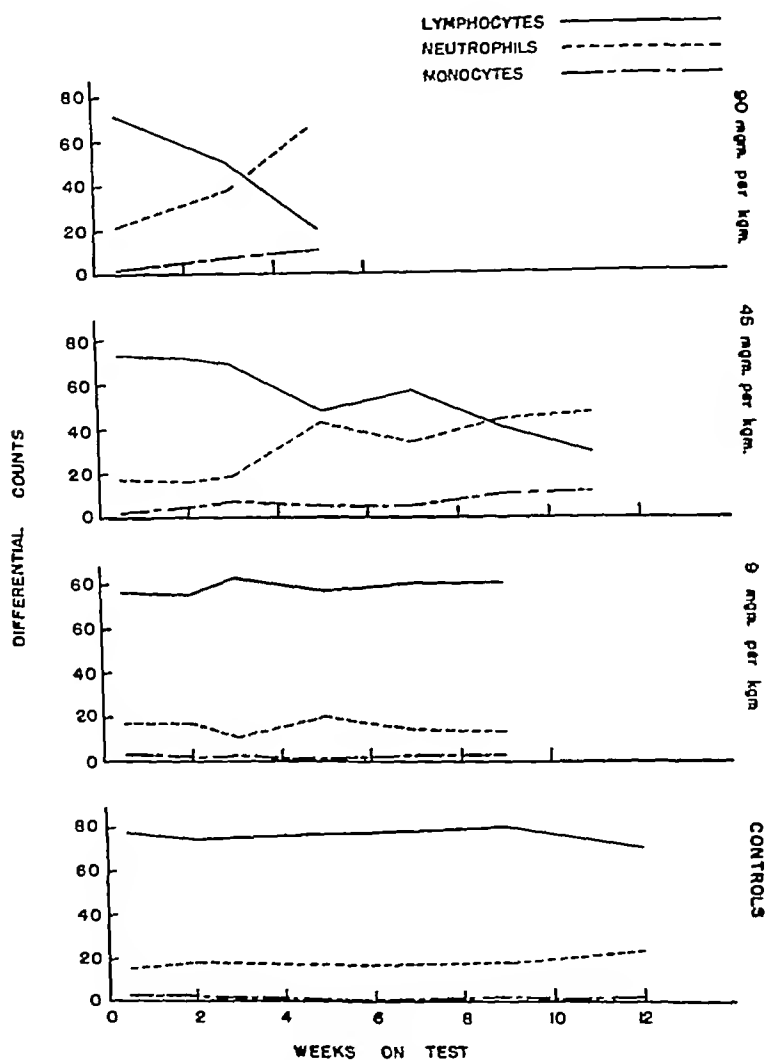


FIG 3 DIFFERENTIAL CELL COUNTS IN RATS DOSED ORALLY WITH QUINACRINE
Each curve represents the average counts in 10 animals

week. The mice showed a polynucleosis and lymphopenia by the sixth day. No inclusions nor changes in differential counts were observed in mice receiving doses of 90 mg per Kg or less.

Rabbits Lymphocytic inclusions were found within 1 to 3 weeks in rabbits dosed orally with 45 or 90 mg per Kg. The animals on the higher dose level died within 8 to 13 days, while a few of those receiving the lower level survived several months.

of drug therapy The total leukocyte counts increased or decreased, but the differential counts remained essentially normal No erythrocytic inclusions were observed, and only a questionable decrease in erythrocyte count or hemoglobin occurred in a few rabbits

Guinea-pigs The guinea-pigs receiving 225 mg per Kg died within 9 days At 1 week, lymphocytic inclusions were observed in these animals Three of the 6 animals dosed with 90 mg per Kg showed lymphocytic inclusions within 9 to

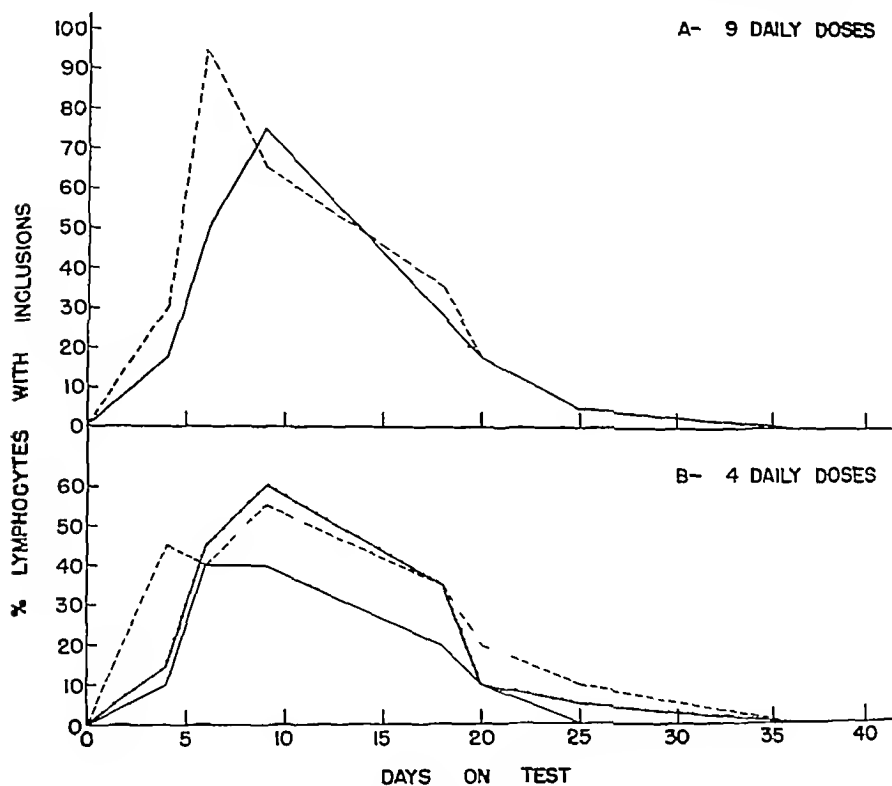


FIG 4 CURVES OF REPRESENTATIVE RATS SHOWING TIME OF APPEARANCE AND DISAPPEARANCE OF LYMPHOCYTES WITH INCLUSIONS

Rats received oral doses of quinacrine—10 per cent L D 50 (90 mg per kg)

16 days, but no other blood changes became evident during the experiment Blood values remained normal in the guinea-pigs dosed with 45 mg per Kg for periods up to 66 days

Hamsters The hamsters dosed with 900 mg per Kg died on the second day They showed lymphocytic inclusions, a decrease in lymphocytes, and an increase in neutrophils just before death In the hamsters which received 225 mg per Kg, lymphocytic inclusions and a slight to moderate lymphopenia and polynucleosis were noted within two days No changes in erythrocyte or total leukocyte counts became evident in animals dosed with 90 mg per Kg for 16 to 29 days All had

lymphocytic inclusions within 7 to 16 days. Shortly after this time inclusions were seen. Quinacrine at a level of 45 mg per Kg failed to produce any marked blood changes in hamsters dosed for 163 days. One animal showed a decrease in lymphocytes and an increase in neutrophils after 16 doses, but the differential count returned to normal and remained so thereafter.

Dogs Doses of 100 mg per Kg or above caused vomiting, salivation, and even death. Dogs dosed six times weekly with 50 mg per Kg showed lymphocytic inclusions within 36 to 49 days. The number of lymphocytes involved was low, never exceeding 3 per cent. With additional dosing, the presence of lymphocytic inclusions was not a constant finding. Most of the dogs developed an anemia and one showed a decrease in leukocyte count from 15,120 to 3,060. The animal showing the leukopenia also had a lymphopenia and polynucleosis, but no differential changes were evident in the other dogs. One dog dosed with 50 mg per Kg vomited frequently, and showed as a result a definite hemoconcentration. When the dose level was reduced to 5 mg per Kg, the blood values returned to normal and remained so throughout the following 8 months, during which period the animal received 106 doses of drug. An anemia and concomitant increased sedimentation rate developed in dogs given 30 mg per Kg for 5 months.

Blood coagulation time, prothrombin and icteric index values remained normal in all dogs regardless of dose or period of dosing.

Monkeys The administration of 100, 200, or 300 mg per Kg caused lymphocytic inclusions to appear in the monkey within 2 to 5 weeks. As many as 39 per cent of the lymphocytes were thus affected. Anemia, which occasionally was only transient, was observed after doses of 20 to 300 mg per Kg. While a leukopenia occurred in some of the animals, no persistent changes in the differential count were seen.

Chickens Chickens getting 900 or 225 mg per Kg showed lymphocytic inclusions after 4 to 9 doses. The birds receiving 90 mg per Kg had, after 48 doses, an average of 2 per cent of the lymphocytes with inclusions. No other blood changes were seen.

Ducks Lymphocytic inclusions appeared within 2 months in ducks receiving 90 or 45 mg per Kg. No other abnormalities were observed in any of the animals.

COMMENT

The oral administration of quinacrine to rats, mice, rabbits, hamsters, guinea-pigs, dogs, monkeys, chickens, and ducks resulted in definite hematological changes. These consisted of anemia, leukocytosis, the appearance of peculiar basophilic inclusions in the cytoplasm of lymphocytes and erythrocytes, and alterations in the differential count. The appearance of lymphocytic inclusions after quinacrine therapy was a constant feature in all species, whereas the other changes varied with the species. Lymphocytic inclusions were usually associated with an increased vacuolation of the affected cells. The percentage of the lymphocytes involved showed a relationship to the dose level and the number of doses of atabrin given. Although lymphocytic inclusions were seen in all nine species, erythrocytic inclusions were observed only in rats, mice, and hamsters. The presence of inclu-

sions in the lymphocytes may be related to the destruction of this cell type since the rat, which showed the most marked lymphopenia, likewise had the highest percentage of lymphocytes with inclusions. Anemia was observed in the rat, monkey, and dog. Scudi and Hamlin¹¹ recently have reported an increase in plasma fibrinogen of dogs fed quinacrine. The increased sedimentation rate seen in dogs in the present investigation is probably the result of both anemia and increased plasma fibrinogen, since both these blood changes are said to increase the rate of settling of erythrocytes.^{12, 13}

Leukocytosis was noted in the rat and leukopenia in the monkey and occasionally in the dog. The usual differential change consisted of a lymphopenia and polynucleosis, while the rat showed, in addition, a monocytosis. This species variation in the effect of quinacrine on the blood picture is scarcely surprising since there is such a wide range among the various species with regard to the lethal toxicity of the drug.

The possibility that some of the blood changes might be due to the activation of a latent infection, such as that caused by *Bartonella muris* or a related organism, was considered. The noninfectious nature of the quinacrine inclusions is indicated by the fact that they could not be propagated in weanling rats, adult splenectomized rats, or young rabbits by the injection of blood from quinacrine-dosed rats containing these inclusions. Furthermore, the inclusions could be maintained in the blood only by the continued administration of quinacrine, cessation of quinacrine dosing resulted in their disappearance. The quinacrine inclusions are unlike the *Eperythrozoon coccoides* inclusions described by Schilling¹⁴ and Elliot and Ford¹⁵ or the gametocyte of *Hepatozoon muris* described by Miller.¹⁶ No *Bartonella* inclusion bodies were found at any time in the red blood cells of rats receiving quinacrine. We have presented elsewhere evidence that the lymphocytic inclusions are probably quinacrine or a substance related to it.⁶

Blood changes similar to those observed after the administration of quinacrine have been reported by Suzuki, Sasaki, and Kurisu¹⁷ in rabbits dosed with trypanflavine. Trypanflavine, like quinacrine, is an acridine dye. After injections of this drug, the authors observed a definite anemia and a leukocytosis followed by a leukopenia just before death. The pseudocoinophils at first increased and then decreased below the initial level. Lymphopenia and monocytosis also developed, but inclusions are not mentioned.

Although aplastic anemia and agranulocytosis have occurred in rare instances in man after prolonged suppressive medication with quinacrine,¹⁸ usually no significant changes in the blood picture are seen during such treatment.^{19, 20} Since hematological studies in human beings dosed with quinacrine are relatively few, there remains the possibility that more complete investigations may reveal other alterations in the blood picture. However, the prophylactic dose of quinacrine for man (100 mg per adult daily, or an average of about 1.4 mg per Kg) is much lower than the dose which produces blood changes in animals. Doses higher than this or very prolonged administration of the drug might result in alterations of the hematological picture in man similar to those observed in laboratory animals.

SUMMARY

The administration of quinacrine to rats, mice, rabbits, hamsters, guinea-pigs, dogs, monkeys, chickens, and ducks results in definite hematological changes. Peculiar basophilic inclusions, usually associated with vacuoles, appear in the cytoplasm of lymphocytes of all nine species following drug therapy. Inclusions of a similar nature are seen in erythrocytes of the rat, mouse, and hamster and rarely in monocytes of the rat.

The rat shows an anemia, polynucleosis, lymphopenia, and monocytosis after quinacrine dosing. None of the other species develop a leukocytosis or monocytosis, but they sometimes show a polynucleosis and lymphopenia after quinacrine therapy. Leukopenia occurs in the monkey and occasionally in the dog following the use of this drug.

ACKNOWLEDGMENT Grateful acknowledgment is due to Drs. H. Molitor and H. A. Charipper, who so willingly gave of their time in discussing this problem and in offering suggestions and criticisms.

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SPECIFICITY OF ISOAGGLUTININ RESPONSE FOLLOWING INJECTION OF GROUP SUBSTANCES INTO GROUP O INDIVIDUALS

Bj WILLIAM C. BOYD, PH D., AND FRANCIS C. LOWELL, M D

IT HAS been shown^{5, 2} that injection of mixed A and B substances prepared from animal sources by Witebsky's method⁷ into individuals of group of A or B will, in a good percentage of cases, cause a sharp rise in the anti-A or anti-B isoagglutinin titer, depending on the blood type of the individual injected. This rise in titer is usually associated with a marked increase in the avidity (rapidity and strength of reactions on slides) of the serums of the injected individuals. There is reason to think that the avidity of a serum is as important a characteristic from the practical point of view as is its titer, although the latter is doubtless a better measure of the amount of antibody present. An individual of group A does not produce any demonstrable anti-A following the injection of A substance, nor does a group B individual produce any demonstrable anti-B after the injection of B substance. In other words, the iso-immune agglutinins produced conform to Landsteiner's rule.⁵ This could be explained by assuming that anti-B agglutinin produced in a group B individual was absorbed by the individual's own red cells or tissues, but it is perhaps more probable that a group B individual is entirely incapable of forming anti-B agglutinin.

No one has been able to show to date that the normal production of anti-A and anti-B isoagglutinins requires that the individual be exposed to the corresponding group substances. Furuhashi³ proposed the theory that the blood group genes cause the production, not only of the corresponding agglutinogen, but of an agglutinin or agglutinins for the group substances not produced. Thus, an individual of group O, according to Furuhashi's theory, produces anti-A and anti-B because of the O genes he possesses. If this theory were true, the question would arise as to whether the amounts of anti-A and anti-B can vary independently in a given group O individual.

At the suggestion of Dr. J. F. Enders, we have investigated the specificity of the antigenic response, using group O volunteers, whose plasma contains both anti-A and anti-B. In these experiments we injected A substance prepared by Witebsky's method from hog stomach⁷ and A and B substance prepared from horse stomach.* The A substance was assumed to be free from B reactivity, but certain considerations have led us to believe that a small amount of B reactivity may reside in the A substance we used (see below). We recognize that, although the A substance used by us will precipitate specifically with high titered naturally occurring human anti-A serum, it still may not be identical with that present in the cells of human beings. The B substance prepared from horse stomach appears to have nearly always associated with it some A activity, possibly as a part of the same molecule.

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* The A and B substances were kindly supplied by Eli Lilly and Co.

No volunteer was accepted who gave a history of allergy, even though we knew nothing about group substances to suggest that their injection into allergic individuals would have been dangerous. So far as could be determined, none of the subjects had received any previous injections of group substances, plasma, or whole blood with the exception of subject 1, who had received a transfusion of blood of his own type (group O) three months before the experiment reported here. A skin test in which 0.02 cc. of the undiluted solution of A substance was injected endermally produced no immediate or delayed reaction in any of the subjects.* The test dose represented an appreciable fraction (one fifth) of the antigenic dose given intravenously and may have contributed to the antigenic response. In view of the number of reaction-free transfusions which have been performed with group O blood conditioned by the addition of 10 mg. of the mixed group substance to each 500 cc. of blood, it did not seem likely that the intravenous injection of the small amount used by us would cause systemic reactions.

After a sample of blood had been obtained, each subject was injected intravenously with 0.1 cc. of a solution which contained 10 mg. group A specific substance per cc. The material was mixed with about 0.9 cc. of sterile saline, for convenience in handling. No chills or other unfavorable reactions occurred. At the end of 14 days, blood was drawn for testing and then some of the subjects received a second injection of 0.1 cc. of a solution which contained 7 mg. of A substance derived from hog stomach and 5 mg. of A and B substance derived from horse stomach in 10 cc. Skin tests with the solution of A and B substance before the second injection gave immediate type wheals and erythema in 4 of 11 subjects. No reactions followed the intravenous injections, however.

The technics of testing have been described elsewhere.¹ Avidities are given in terms of time intervals which increase logarithmically (each longer than the preceding by the factor $\sqrt{2}$), instead of in seconds. This is based on observations¹ that the longer the time which elapses before agglutination is observed, the less important the exact number of elapsed seconds becomes. To take subject 14 as an example, it was found that his serum gave first visible agglutination with A_1 cells in time interval number 5, an interval which began 4.6 seconds after the serum and cells were mixed, and lasted 7.2 seconds. Complete agglutination, however, was not observed until the eighth interval, which began 13.1 seconds after mixing, and lasted 27.5 seconds. The reason for the choice of these mechanically timed intervals is discussed by Boyd¹ elsewhere. We may simply say here that our experience suggests that a difference of one interval has about the same significance all along the scale.

RESULTS

The changes in titer of isoagglutinins following the injection of A substance are shown in table 1. In an effort to present the material as completely as possible, only initial titers (1 c., before injection) are given, followed by the factors by which

* In one of us who was not a subject there developed a wheal, flare, and itching at the site of the endermal injection in about 5 hours. This reaction was similar to that which, on several occasions, had been observed to follow the endermal injection of diluted horse serum.

these must be multiplied to obtain the titer after injection. To take an example, the serum of subject 14 gave a titer of 64 against A_1 cells before injection, and after the injection of A substance, a titer of $64 \times 4 = 256$. After a further injection of A and B substance, this titer went up to $64 \times 4 \times 2 = 512$. Of the 22 subjects receiving a single injection, 16 showed an increase in titer of four-fold or more as measured by A_1 cells and 15 as measured by A_2 cells. There was also some increase in the

TABLE 1—Changes in the Titer of Isoagglutinins Following a First Injection of A Substance and a Second Injection of A and B Substance Two Weeks Later

Subject number	As tested with A_1 cells			As tested with A_2 cells			As tested with B cells		
	Titer before injections	*Increase in titer after injection of		Titer before injections	Increase in titer after injection of		Titer before injections	*Increase in titer after injection of	
		A substance	A + B substance†		A substance	A + B substance†		A substance	A + B substance†
1	128	8		8	16		16	2	
2	1024	8		128	16		256	2	
3	32	4		4	8		16	2	
4	32	64		32	2		64	—	
5	128	16		128	8		64	2	
6	128	—		64	—		128	—	
7	32	8		8	16		16	4	
8	128	—		32	2		16	2	
9	64	32		64	4		32	—	
10	16	4		8	2		256	2	
11	256	32		128	8		64	4	
12	128	8	2	8	32	—	8	8	8
13	128	4	8	128	—	8	64	2	8
14	64	4	2	32	2	2	64	2	—
15	256	—	—	32	4	—	64	2	2
16	64	—	2	8	8	—	32	—	16
17	32	4	8	16	8	2	32	—	8
18	64	8	—	16	16	—	64	—	8
19	32	8	—	16	8	—	64	—	—
20	512	—	8	128	4	2	128	2	2
21	32	8	2	32	8	—	16	8	—
22	128	—	2	32	2	2	64	—	2

* Ratio of new titer to original titer. Dilutions made in steps of two

† Further increase in titer after second injection

A dash (—) means no increase in titer (i.e. a ratio of one). When no entry appears, the test indicated was not done.

titer as measured by B cells, 4 subjects showing a four-fold rise or more and only 7 showing no change at all. Thus as measured by A_1 or A_2 cells approximately two thirds of the subjects showed a four-fold rise in titer or more whereas less than one fifth showed such a rise for B cells. In every instance in which a rise in the anti-B titer occurred there was an equal or greater rise in titer for either A_1 or A_2 cells or both. In subjects 4, 9, 16, 17, 18, and 19 a significant increase in titer for either A_1 or A_2 occurred in the absence of any change whatever in the titer for B cells. The in-

intensity of the antigenic response varied widely in different individuals. Subject 6 showed no change whatsoever, and subjects 8 and 22 showed no more than a two-fold increase in titer with any of the three types of cells, changes which are of doubtful significance. On the other hand, as measured with A_1 cells, subject 4 showed a 64-fold rise, and subjects 9 and 11 showed a 32-fold rise as did also subject 12 as measured with A_2 cells.

Changes in titer for A_1 and A_2 cells did not increase to the same degree in each instance. In 12 subjects the increase in isoagglutinins, measured with A_2 cells, was greater than that measured with A_1 cells, whereas the reverse was true in 8 subjects. In subjects 15, 16, and 20 a four-fold or greater increase in titer for A_2 cells occurred in the absence of any change in titer for A_1 cells, and the reverse was true for subject 13. A relationship between the initial titer and the intensity of the antigenic response is perhaps seen in the titers for A_1 cells. Of 7 subjects (3, 4, 7, 10, 17, 19, and 21) who had initial titers of 1/32 or less, all had a four-fold or greater increase. Of the remaining 15 subjects, all of whom had an initial titer of 1/64 or more, only 9 showed a four-fold or greater rise.

The titers of isoagglutinins for A_1 or A_2 cells in the serums of subjects 12 to 22, two weeks after receiving the injection of A substance, were not affected by absorption with B or O cells.

As mentioned before, 11 subjects received a second injection consisting of a mixture of A and B substances two weeks after the first and were again bled after a period of two weeks. The changes in titers for A_1 , A_2 , and B cells are also shown in table 1. A further significant increase in titer for A cells occurred in 3 and for A_2 cells in 1, a definitely smaller proportion than that following the first injection. Against B cells, on the other hand, 5 subjects showed a four-fold or greater increase, 3 of them (subjects 12, 16, and 18) showing no significant change in the A_1 or A_2 titer. Subjects 16 and 18 had responded specifically to the previous injection of A substance.

Changes in avidity were also followed in 15 of the 22 subjects receiving a single injection of A substance and in 11 subjects receiving a second injection of A and B substance. The results are shown in table 2. Of 10 subjects (2, 3, 7, 12, 13, 14, 17, 18, 19, 21) whose serums showed an increase in the titer of A_1 cells of four-fold or more following the injection of A substance (table 1), 6 (3, 7, 12, 13, 14, 21) showed a significant increase* in avidity for A_1 cells and 4 showed no significant change. On the other hand, of 5 subjects (6, 15, 16, 20, 22) who showed no significant increase in isoagglutinins for A_1 cells, only 1 (20) showed an increase in avidity. As tested with A_2 cells the correlation of change of avidity and increase in isoagglutinin was slightly better. Of 11 subjects (2, 3, 7, 12, 15, 16, 17, 18, 19, 20, 21) in whom a significant increase in isoagglutinins occurred only 3 (2, 15, 16) failed to show an increase in avidity. Of 4 subjects (6, 13, 14, 22) showing no significant increase in isoagglutinins, 2 (6, 22) also showed no increase in avidity. Following the injection of A substance the agglutinins for B cells rose significantly in 3 (7, 12, 21) of the 15 subjects in whom tests for avidity were made. In these 3 subjects the avidity for B

* A decrease of 2 or more logarithmic intervals¹ is considered significant in this discussion.

cells also increased. In 6 subjects (6, 16, 17, 18, 19, 22) in whom absolutely no change in the isoagglutinin titer for B cells took place there was a significant increase in avidity in 2 (6, 16).

Following the second injection which contained both A and B substances the increases in avidity were more striking, especially as measured with A₁ and B cells (The increase in avidity had been most marked following the first injection when measured with A₂ cells.) However, correlation with changes in titer of isoagglutinins was almost entirely lacking when the avidity was tested with A₁ or A₂ cells.

TABLE 2.—*Isoagglutinin Avidities in Group O Serums before and after Intravenous Injection*

Donor number	Avidities ' before injection			Avidities after injection of A substance								' Avidities after injection of A + B substance		
	A ₁ I C	A I C	B I C	A ₁ I C	A ₂ I C	B I C	After absorption with				A ₁ I C	A ₂ I C	B I C	
							B cells		O cells					
							A ₁ I C	A ₂ I C	A ₁ I C	A ₂ I C				
-	4-7	4-7	5-9	3-6	3-6	3-6								
3	3-8	4-8	5-8	3-6	3-6	3-6								
6	3-6	4-7	5-9	4-7	4-7	4-7								
7	3-8	3-8	5-9	3-6	3-6	3-6								
12	4-8	4-9	5-9	4-6	3-6	3-8	1-3	2-4	2-3	2-4	2-4	-5	-5	
13	3-8	4-9	4-7	3-6	3-6	3-7	-4	3-5	-3	2-4	2-4	2-5	2-5	
14	5-8	5-9	5-8	3-7	3-6	3-7	2-4	2-5	2-3	-4	1-4	-5	2-6	
15	3-7	4-7	5-8	4-7	3-7	3-8	2-5	3-7	2-5	3-7	1-5	2-5	-5	
16	3-8	4-8	4-9	3-8	5-8	-8	2-4	3-6	-3	2-5	2-5	2-6	1-4	
17	4-8	5-9	4-7	4-7	3-7	3-8	2-6	3-7	-4	3-6	1-5	-7	1-4	
18	3-7	3-8	3-7	4-6	3-6	3-7	3-4	3-5	-5	3-5	1-4	-4	1-4	
19	3-7	3-8	3-7	3-6	3-6	3-7	2-5	3-6	-4	3-5	1-5	-6	1-6	
20	3-6	3-6	3-6	3-4	3-4	3-4	2-3	-4	-3	-4	1-3	1-3	2-5	
21	4-7	4-8	4-13	1-6	2-6	3-6	3-4	3-5	-4	3-5	-4	-5	2-7	
22	3-7	3-7	4-7	4-6	3-7	4-7	3-5	3-6	3-4	3-6	2-6	-6	-4	

Avidities are recorded in terms of intervals of time required (on an arbitrary logarithmic scale [1]) for agglutination to be seen (I) and for it to become substantially complete (C).

Significant increases in avidity were, however, associated with significant increases in isoagglutinins for B cells in subjects 16, 17, and 18.

The specificity of the observed changes in avidity is open to question. When some of the serums were absorbed with O and B cells (table 2) and were then tested with A₁ and A₂ cells, an increase in avidity was observed to have taken place in most of the serums. As mentioned before, absorption of these serums with O and B cells did not alter the titer for A₁ and A₂ cells.

DISCUSSION

From the data obtained in this study it seems reasonable to conclude that in certain individuals the production of isoagglutinin for one group substance can occur entirely independently of the production of the other. Caution is necessary,

however, because the group substances injected may not be identical immunologically with those occurring naturally in human cells, and therefore the possibility cannot be ruled out that in each instance an antibody differing from the naturally occurring isoagglutinins was being produced *de novo*. However, nothing in this study indicates that the antigenic response involved anything other than an increase in the titer of true isoagglutinins.

That some B activity was present in the supposedly pure A substance used in this study is suggested by the increase in anti-B which followed the injection of A substance in some subjects and also by *in vitro* experiments designed to test just this point. When A substance was added to serums containing A and B agglutinins in amounts sufficient to abolish all anti-A activity in the serum, a definite decrease in the titer of anti-B activity took place. This change was greater than that which could have been accounted for by mere dilution. Thus the rise in anti-B titer which occurred unequivocally in 10 of the 22 subjects receiving an injection of pure A substance may well have been due to the presence of small quantities of B activity in the preparation. A second possibility is that the injection of A substance causes in some individuals an increase in other related or unrelated antibodies. Therefore, the occasional rise in anti-B titer following the injection of the A substance may carry no weight as an argument against the theory that the production of the two isoagglutinins occurs independently in all individuals.

One may conclude from this study that an increase in the titer of anti-A can occur without any increase in anti-B. The presence of traces of B activity in the A substance used, nonspecific stimulation of antibody production, or both may explain those instances in which a rise in anti-B occurred following the injection of A substance. There is also the rather unlikely possibility that the observed independent production of anti-A can occur only in certain individuals and that in others the production of one isoagglutinin necessarily entails production of others.

The tests for the avidity of the isoagglutinins were carried out in the hope that they would shed further light on the specificity of the antigenic response to the injection of group substances. However, the avidities were influenced by factors the nature of which was not determined. Although increases in avidity usually took place with increases in the titer of isoagglutinins, such changes in avidity could not be correlated with changes in titer alone.

SUMMARY AND CONCLUSIONS

The anti-A and anti-B titers in group O individuals may vary independently following injection of group-specific substances, although such independent variation was not always seen. The rise in anti-B which followed the injection of A substance in some instances may have been due to traces of B activity in the A substance or to nonspecific stimulation of antibody which may follow the injection of any antigen. The part played by such factors was not determined.

It is concluded that the presence of A and B agglutinins in O individuals is not dependent on a single cellular characteristic responsible for the simultaneous formation of the isoagglutinins.

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THE BLOOD GROUPS, SUBGROUPS, AND RH FACTOR OF THE MAPUCHE INDIANS OF THE PROVINCE OF CAUTÍN, CHILE

By L. SANDOVAL S., M.D., C. HENCKEL, M.D., AND L. GIVOVICH, M.D.

SINCE our first work on blood groups (1929) we have been desirous of applying these studies to the sero-anthropology of the Indians of our country. Bernstein,¹ Mazza and Franke,² Moss and Kennedy,³ Nigg,⁴ Snyder,⁵ Coca and Deibert,⁶ and others studied different aspects of the sero-anthropology of American Indians. In 1930, Onette and Castillo⁷ published an interesting work on Aurarian blood groups. Then came the work of Allen and Scheffer,⁸ on the classic blood groups, and of Allen and Larsen on the M and N types.⁹ We shall only mention, because we do not propose to do a critical or bibliographical study on the subject, the names of Arce Larreta, Battistini, Candela, Favero, Ferreira, Gates, Golden, Gonzalez, Goodner, Grant, Hirszfeld, Kahn, Kossovitch, Landsteiner, Levine, Marroquin, Matson, Paulotti, Ribeiro, Ridt, Rife, de la Rivere, Santiana, Schiff, Boyd, Wyman, and Wiener. We do not include Rham's studies, notwithstanding the fact that he made an investigation in Mapuche, Fueguino, and also on Pascuense Indians, because we believe that these investigations require verification by more accurate material and methods. First we studied the mean values of groups, subgroups, types, and factors in the population of the capital city, Santiago, where Indians are rare. We then began a series of investigations¹⁰⁻²¹ on blood groups, subgroups, types, and the Rh factor in various subjects. Henckel, Castelli, and dal Borgo²² investigated (1941) the mean values of M and N types on 100 Mapuches from the Province of Cautin, with serums prepared by our assistant Varleta in the Laboratorio de Policia Tecnica, and controlled with North American serums from Landsteiner.¹²

In November, 1945, with Henckel and Givovich, we went to Temuco, capital city of the Province of Cautin (thanks are given to D. Gen. de Investigaciones for help rendered to us), and there performed serological investigations on 205 Mapuches, without white crossing, all of whom the morphologist-anthropologist Henckel found to correspond with the type of Indian from this region. We visited the elementary schools of Padre las Casas, Metrenco, Cajón, Labranza, and Boroa, the farm school at Cajón, The Infantry Regiment at Tucapel, and the Temuco hospital and jail. Thanks are due to authorities and teachers from Temuco and the other localities for their help.

The 205 individuals investigated were 4 to 60 years old, 42 were women and 163 men. We used three anti-A and anti-B human serums, which were retested daily. The titers of the serums were 1/520 and 1/1024. The slide technic was used in the field, where we took the samples, the tests were later checked with the

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tube technic, with a 2 per cent suspension of erythrocytes (0.85 per cent NaCl) in the Central Laboratory (Seguro Obrero) of Temuco

The subgroups were tested with two anti-A serums, one prepared by our assistant Varleta and the other obtained from Blood Donor Service, Jamaica, New York, and standardized by Wiener. The standard Rh factor was also investigated with two serums, one prepared by Givovich, and the other of North American origin. Thanks are due to our colleague Prof. Dr. Vaccaro H. who kindly gave us 2 cc of American Anti-Rh Standard Serum to complete our series. The subgroups were done on slides and tubes, and the Rh factor in test tubes using the regular technic, as described by Wiener and Levine.

All reactions in tubes were performed in an incubator at 37° C.

RESULTS

The condensed data for the 205 Mapuches are as follows

TABLE I

Group and Subgroup	O	A1	A2	B	A1B	A2B	Total
Absolute no	178	17	1	7	2	0	205
Per cent	86.8	8.3	0.5	3.4	1.0	0	100

If we use the Thomsen and Wellisch formula, we have

$$P_1 = \sqrt{O + \bar{A}_1 + \bar{A}_2} - \sqrt{O + \bar{A}_2}$$

$$P_2 = \sqrt{O + \bar{A}_2} - \sqrt{O}$$

$$q = \sqrt{O + B} - \sqrt{O}$$

$$r = \sqrt{O}$$

Substituting the frequencies obtained

$$P_1 = \sqrt{9559} - \sqrt{8730} = 4.3\%$$

$$P_2 = \sqrt{8730} - \sqrt{8682} = 0.3\%$$

$$q = \sqrt{9023} - \sqrt{8682} = 1.8\%$$

$$r = \sqrt{8682} = 93.2\%$$

Therefore, $P_1 + P_2 + q + r = 99.6$ per cent, which corresponds satisfactorily with the theoretical expectancies. The Rh standard factor was present in 202 individuals and absent in 3 cases. We have, then

$$\text{Rh} + = 98.58 \text{ per cent, Rh} - = 1.42 \text{ per cent}$$

Of the 202 cases which were Rh positive, 14 were weakly positive with the standard anti-Rh serums. It is possible that these belonged to different Rh subtypes, but without the necessary antisera it was impossible to settle this problem. At some future time we hope to have these antisera for determining the Rh subtypes.

If we give our results in percentages, including for comparison the results obtained in Santiago, we have¹⁶⁻¹⁸

TABLE 2

Place	Number tested	A1	A2	B	A1B	A2B	O	P1	P2	Q	R
Santiago (whites)	2000	25 95	3 85	9 95	2 25	0 25	57 75	15 08	2 48	6 28	76 0
Cautin (Mapuches)	205	8 29	0 48	3 41	0 96	0 00	86 82	4 33	0 27	1 80	93 17

The Indians examined show little crossing with whites because they have a high percentage of O, with small frequency of A1 and A2. The A2 subgroup was present only in one case. The frequency of Group B is 3.41 per cent in our series. This might be explained, as Landsteiner, Wiener, and Matson suggest, by crossing with whites, or perhaps the Mapuche Indians have a small frequency of blood group B.

Comparing the distribution of the Rh factor among our 205 Mapuches with the data from the studies in Santiago, we have

TABLE 3

	Number tested	RH+	RH-
Santiago whites ²¹	2,342	90 55	9 35
Cautin (Mapuches)	205	98 58	1 42

We did not have a 100 per cent Rh factor frequency as in Landsteiner, Wiener, and Matson's studies on American Indians, but we must draw attention to the number of subjects tested, the possible crossing with whites (if we remember the percentage of blood group B), and also that the 100 per cent Rh might not apply in other Indian populations even though full-blooded.

Our series is relatively small, but tests in 1000 cases, which we believe to be the best number for a reliable investigation, are very difficult to obtain in Chile, because the number of Indians is small and very few of them are full-blooded. Similar difficulties have been present in all other investigations made on the American continent with Indian tribes which are at present nearly extinct. The situation is somewhat different in Ecuador, Peru, Bolivia, Brazil, etc., where the Indians are numerous and many are of pure blood.

SUMMARY

The authors present a sero-anthropological study of 205 Mapuche Indians from Cautin (Chile). There were 86.8 per cent of group O, 8.3 per cent of subgroup A1, 0.5 per cent of subgroup A2, 3.4 per cent of group B, and 1.0 per cent of subgroup AB.

The 3.4 per cent group B in the series may be due to crossing with whites or to the fact that this group was always present in Mapuches even before contact with whites.

The frequency of the standard Rh factor percentage is very high (98.6 per cent) but not 100 per cent as in the data of North American investigators.

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EDITORIAL

THE HEMORRHAGIC DISORDERS

FOR purposes of convenience, and from the standpoint of our present-day knowledge, it may be assumed that there are three mechanisms which are concerned in preventing the loss of blood from the circulation into the tissues. These are the capillary wall or endothelial lining, the blood platelets, and those chemical or enzymatic substances within the circulating blood which are concerned with blood coagulation. A breakdown in any one of these several mechanisms may result in seepage of blood and thus in petechiae and ecchymoses, in continued oozing from small blood vessels, or in the lack of clot formation. Based on this perhaps oversimplified classification, one may distinguish three types of hemorrhagic disorders.

(1) Vascular or nonthrombocytopenic purpuras having nothing to do with either the morphologic or chemical elements of the blood, (2) thrombocytopenic purpura, largely associated with a great reduction in platelets, and, (3) such disorders as hemophilia, hypoprothrombinemia, etc., in which a plasma factor is concerned.

Aided by the appropriate laboratory tests, this classification becomes useful in the diagnosis of most hemorrhagic disorders. Cases are occasionally seen, however, which are rather more difficult to classify. Such are the instances of bleeding disturbances which are associated with an increased bleeding time in the presence of a normal platelet level and a usually normal tourniquet test. For these cases, the designation of *pseudohemophilia* has in recent years been utilized.

Pseudohemophilia, admittedly a poor designation, is often hereditary although isolated cases are common. Minor operations lead to excessive and at times uncontrollable bleeding. As MacFarlane has stated, there appears to be a disturbance in the retractility of the capillaries following trauma, as a result of which continued oozing of blood takes place.

In some respects severe cases of the disorder are more difficult to treat than the fundamentally more serious diseases of hemophilia and idiopathic thrombocytopenic purpura. In the former, transfusions of blood or the use of antihemophilic plasma globulin are usually effective, and in thrombocytopenic purpura, removal of the spleen usually cures. In *pseudohemophilia*, on the other hand, transfusions and splenectomy are of no value, and unless the bleeding points can be gotten at locally and packed with thrombin-fibrin foam, the bleeding may remain completely uncontrolled. Ascorbic acid, vitamin P, and the more recently described rutin still leave much to be desired in the treatment of disorders of capillary permeability and retractility leading to undue bleeding.

WILLIAM DAMESHEK, M D

ABSTRACTS

HEMATOPOIETIC TISSUES

OLIVER P JONES, Ph D

CHANGES IN THE BLOOD OF THE DOG WITH AGE *H E Ederstrom and B DeBoer Anat Rec 94 663-70, 1946*

Owing to the increasing number of medical research problems which require experimentation on dogs it is very fitting that a well controlled study of dogs blood be presented at this time. The results reported in this article were obtained from studies on 158 normal mongrel dogs ranging in age from 1 day old to adult. The red blood cell count, hemoglobin values, and hematocrit readings decreased steadily during the first 2 to 3 weeks after birth, then they gradually increased until most dogs were 6 months old—when the hematopoietic system was stabilized in the adult form. The blood specific gravity readings followed the general trend of the red blood cell changes. Mean corpuscular volume and mean corpuscular hemoglobin values decreased after the first 3 days of life to approach the adult figure at about 4 weeks of age. Only minor changes in the mean corpuscular hemoglobin concentration were noted with age.

THE PRESCAPULAR LYMPH NODE OF THE OX AND ITS RELATION TO LYMPHATIC DRAINAGE OF THE SKIN *W M Henderson J Anat 80 107-10 1946*

Although the present article is of great importance in veterinary medicine, there are certain general principles which might very well obtain in the human being. In order to study the fate of intracutaneous injections in the ox and the lymphatic drainage of the skin, either India ink or a 1 per cent aqueous solution of trypan blue was injected in amounts ranging from 0.1 to 10.0 cc. The cattle were killed at intervals varying from a few seconds to 24 hours after injection and the prescapular lymph node removed. Four skin areas of the ox were associated with drainage to particular portions of the prescapular lymph node. These skin areas were the cranial third of neck, the scapular region, the ventral aspect of the neck and brisket and the forelimb from distal humerus to the carpus. Lymph drainage from each of these four skin areas was confined to a particular portion of the lymph node. This knowledge is of importance in veterinary medicine because the prescapular lymph node easily palpable in the living and accessible in the dead animal, receives afferent vessels from that part of the body most commonly used for intracutaneous and subcutaneous injections.

THE AMOEBOID MOVEMENT OF THE MAMMALIAN LEUCOCYTE IN TISSUE CULTURE *P P H De Bruyn Anat Rec 95 177-92, 1946*

For a number of years it has been known that amoebae move as a result of reversible sol gel changes. Leukocytes have been observed to have similar changes when they migrate and in addition, Lewis (1942) reported the presence of constriction rings which remain stationary as the cell moves. De Bruyn reports the results of studying these changes in tissue cultures of rabbit lymph nodes and bone marrow. The leukocyte movements were recorded with time lapse photographs on reversible film at the rate of 60 per minute. Constriction rings were observed in cells regardless of whether they had the handmirror or wormlike types of motion but are much more frequent in the latter. If a given cell had a constriction ring at a certain place in the culture, other cells passing this point would also have a constriction ring as they too passed this place. This suggested that these rings were caused by physical obstacles within the culture medium. Similar results were obtained when leukocytes moved on the flat surface of the cover glass. From these observations it was concluded that constriction rings do not play an essential role in the movement of cells. Lateral protuberances on the body of the cells were observed to be more or less stationary rather than waves. These lateral protuberances were formed when the anterior pseudopodial area became immobilized, and they disappeared when the tail gradually approached this area. These pro-

tubercances are probably due to a superficial plasma gel formation which may contract and drive the cell forward. The possible mechanisms concerned with plasma sol and plasma gel changes are discussed.

BLOOD TRANSFUSIONS AND ERYTHROCYTE SURVIVAL

CHARLES P. EMERSON, M.D.

SURVIVAL OF NORMAL ERYTHROCYTES AFTER TRANSFUSION TO PATIENTS WITH FAMILIAL HAEMOLYTIC ANAEMIA (ACHOLURIC JAUNDICE) *J. V. Dacie and P. L. Mollison* *Lancet* 1: 550-52, 1943

The authors studied the fate of transfused normal red cells administered to six patients with congenital hemolytic jaundice manifested by chronic anemia, hyperbilirubinemia, reticulocytosis, spherocytosis, and increased osmotic erythrocyte fragility. The rate of destruction of the donor cells following transfusion was determined by means of serial red counts, employing the selective agglutination technic of Ashby when group O cells were received by individuals of other blood groups or the method of Wiener utilizing differences in the M and N grouping. In similar fashion the survival of red cells obtained from one of these patients before and after splenectomy was determined after injection of the blood into individuals with mild secondary anemia. The purpose of the investigation was to discover whether the excessive blood destruction associated with congenital hemolytic jaundice is explainable on the basis of an abnormal mechanism of blood destruction in which a circulating red-cell antibody or pathologic splenic function might be operative, or whether the red cells are inherently defective and consequently short-lived.

It was found that normal erythrocytes injected into five patients with congenital hemolytic jaundice survived as long as in recipients without hemolytic disease; the survival periods totaling from 100 to 130 days. In one patient complete destruction of donor cells occurred within 60 days, in this instance, however, an immunologic hemolytic mechanism could be implicated, namely, the stimulation of anti Rh iso-antibodies in response to the transfusion of Rh positive blood.

The reverse experiment, in which one of these patients served as a blood donor for a patient with iron deficiency anemia, demonstrated a marked susceptibility to destruction on the part of congenital hemolytic jaundice red cells. Blood samples obtained prior to, and one year following splenectomy were destroyed at comparable rates, disappearing completely from the recipients' blood within 14 and 19 days respectively.

Similar studies were carried out in a patient with paroxysmal nocturnal hemoglobinuria in whom transfused red cells survived in normal fashion, providing confirmatory evidence that the mechanism of excessive blood destruction in this disease, as in congenital hemolytic jaundice, is related to a pathologic property of the red cell.

THE DESTRUCTION OF TRANSFUSED ERYTHROCYTES IN ANAEMIA *G. M. Brown, O. C. Hayward, E. O. Powell, and L. J. Wiggs* *J. Path. & Bact.* 56: 81-94, 1944

Patients with various types of anemia were transfused with red cells from normal group O donors. The survival of the injected cells in these recipients, whose blood groups were other than group O, was thereafter estimated by the Ashby technic.

The rate of donor cell destruction in six patients with anemia due to chronic iron deficiency was relatively constant, the total duration of survival approximating 100 days, the average life span, 50 days. Three patients with clinical evidences of acquired hemolytic disease eliminated the donor cells much more rapidly; the average life of the transfused erythrocytes in these cases ranging from 7 to 13 days. The rate of donor cell destruction moreover did not follow a linear curve, as in patients with iron deficiency, but an exponential type of curve, the rapidity of disappearance apparently being a function of the concentration of donor cells in the recipient's blood. A disappearance curve of this type is interpreted as evidence of an abnormal hemolytic process or iso-antibody operating indiscriminately against all red cells irrespective of their age or origin.

Similar results were obtained in cases with anemia complicating acute infections, in a patient with pernicious anemia of pregnancy and one with multiple myeloma. On the other hand the destruction of donor cells in patients with anemia attributed to chronic infection and chronic nephritis, and in a patient

with pernicious anemia receiving liver therapy, occurred at a normal rate, implying the absence, in these cases, of a hemolytic disorder

SURVIVAL OF TRANSFUSED RED CELLS IN BLACKWATER FEVER CIRCULATION AND OF BLACKWATER RED CELLS IN NORMAL CIRCULATION *H Foy, A Kondr, A Rebelo, and A Socero* Tr Roy Soc Trop Med & Hyg 38 271-86 1945

Blood transfusion has been applied by the authors in studying the hemolytic mechanisms responsible for blackwater fever. Two patients exhibiting this syndrome in the course of chronic malaria and quinine therapy were transfused with normal group O blood. The destruction of the donor cells measured by the Ashby technic, was excessively rapid, as rapid as was the destruction of the recipients' own red cells when transfusions were given during hemolytic crises. During convalescent periods more prolonged erythrocyte survivals were observed, the life span of injected cells eventually becoming normal. Red cells obtained from a patient with blackwater fever during a hemolytic crisis and a second specimen withdrawn 10 days following the episode (5 days prior to another crisis) survived for abnormally brief periods when injected into recipients in whom there were no evidences of hemolytic disease.

The authors conclude that the syndrome of blackwater fever is produced by some unidentified extracellular agent, or factor, capable of destroying more normal donor cells as well as the patient's erythrocytes. Red cells that escape destruction during the acute crisis are nevertheless irreversibly damaged by the hemolytic mechanism, and, whether they continue to reside in the patient's circulation or are transferred to a normal environment, their survival is consequently brief. A direct attempt to demonstrate a circulating hemolysin by injecting 500 cc. of plasma, obtained from a patient during a crisis, into a recipient with active *P. falciparum* malaria receiving quinine, failed to provoke a hemolytic reaction. This failure, as the authors point out, may signify merely that complete exhaustion of the hypothetical circulating hemolysin had already occurred, possibly by cellular adsorption.

MATURATION AND DESTRUCTION OF TRANSFUSED HUMAN RETICULOCYTES. EVALUATION OF RETICULOCYTE EXPERIMENTS FOR THE MEASUREMENT OF HEMOGLOBIN METABOLISM *L E Young and J S Laurence* J Clin Investigation 24 554-63, 1945

An unusually direct and ingenious approach to the problem of reticulocyte maturation is described by the authors, whose methods of investigation included experimental blood transfusion followed by selective agglutination studies of the recipient's blood. A child with aplastic anemia, blood group A received blood from a patient with an atypical hemolytic anemia blood group O. Three hundred and fifty cc. of red cells were injected, 73 per cent of which were reticulocytes. The fate of the mature and of the reticulated donor cells was determined by means of Ashby counts in combination with reticulocyte counts. The data obtained indicated that maturation of the transfused reticulocytes occurred in the course of approximately 140 hours, almost identical findings, with respect to maturation rate, were obtained on *in vitro* incubation of the donor blood in Simmel's solution. Destruction of the donor cells was abnormally rapid, none being detected in the recipient's circulation after 8 days. Nevertheless, the injected reticulocytes appeared to be immune to destruction until they had attained maturity.

It is concluded that, if the average maturation time of reticulocytes is as prolonged as that found in this case, the reticulocyte count must be considered unreliable as a quantitative index of the rate of hemopoiesis, and reticulocyte experiments inadequate as a basis for computing the life span of red cells. Thus, if all reticulocytes mature after an average period of 140 hours in the peripheral blood, and if the average percentage of reticulocytes in the normal blood is 0.7, it follows that the average life span of the normal red cells is 833 days, more than 8 times greater than indicated by transfusion studies or measurements of pigment excretion. The authors regard this discrepancy as evidence that many erythrocytes must leave the bone marrow as mature cells excepting when erythropoiesis is excessively rapid. An alternate explanation might also be hypothesized, namely that reticulocytes may enter the peripheral circulation at various stages of maturity, depending on the rate of blood production, and that completion of their maturation in the peripheral blood may normally require much less than the 140 hour period observed in this case of severe hemolytic disease.

NEWS AND VIEWS

The following letter has been received from Venezuela

Caracas, August 1, 1946

May we bring to your attention the first cases of findings of sickle cell anemia and sicklemia in Venezuela

We think the demonstration of the existence of such blood modifications in our country is of the utmost importance in knowledge of corresponding regional pathology, inasmuch as previous investigations made by other authors were negative

In Venezuela there exists a great hybridization of the people through the mixing of three ethnologic elements—whites, Negroes, and Indians—to such an extent that the individual with racial purity is exceptional, and this is what makes the study of the distribution of the disease more interesting. Moreover, the incidence of cases was relatively high in our first investigations

The identification of 2 cases of sickle cell anemia and 4 cases of sicklemia has been possible. The first cases were found in the Paparo region (Valley of the Tuy, Miranda State). The analysis of 40 nonselected natives permitted the finding of globular deformation in 2 subjects. Then a typical case of sickle cell anemia was found in a 3 year old boy of Cumarebo (Falcón State), who died of intercurrent disease four months after diagnosis was established. Finally, we could demonstrate the existence of drepanocytic anemia in a young man of 17, born in Guama (Yaracuy State), whose two brothers were found to be affected by sicklemia. Attention is called to the origin of these patients, all of whom were born in the coastal zone of the country where the Negro population ranks highest. It is interesting to note, however, that of the 4 cases of sicklemia mentioned, the skin color of 3 cases was dark whereas the remaining was completely white. The 2 cases of drepanocytic anemia occurred in a blond-eyed subject and the other in a hybrid person.

Up to date we do not know if this high incidence of the disease in such a small series analyzed is a matter of coincidence or shows the great frequency of the disease in Venezuela. Much more extensive studies are required to clarify this problem and at the present time we are engaged in this work.

P O Box 1195
Caracas, Venezuela

H Benaim Pinto
L M Carbonell
O L Gómez

An author and a subject index, together with a title page and Table of Contents for all issues of Volume I of BLOOD—The Journal of Hematology, will be sent to all subscribers free of charge. In order not to delay publication of this issue, they will be mailed with the first issue of Volume II.

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